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(54) SYNTHESIS INHIBITOR CONTAINING GINSENOSIDE OF PROTEIN BELONGING TO HSP 27 FAMILY

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the subject HSP47 synthesis inhibitor capable of suppressing collagen synthesis and treating diseases showing the condition of sthenia of extracellular matrix production, comprising a ginsenoside as an active ingredient.
SOLUTION: Ginseng or koji is subjected to extraction treatment with water or an organic solvent and separated from impurities by filtration, centrifugal separation, etc., to give a crude extract, which is optionally purified by an adsorbent to give a ginsenoside (e.g. ginsenoside Rg1). Then, the ginsenoside is pharmaceutically manufactured alone or with an ordinary carrier to give the objective synthesis inhibitor which contains the ginsenoside as an active ingredient, has an inhibitory acton on synthesis of a heat shock protein (HSP47) having 47 kilodalton molecular weight, suppresses collagen synthesis in organs and is useful for treating diseases such as cirrhosis, interstitial lung disease, chronic renal insufficiency, hypertrophy of heart, postoperative cicatrice, burn cicatrice, keloid, scleroderma, arteriosclerosis, arthrorheumatism, etc.

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(54)【発明の名称】　ジンセノサイド類含有ＨＳＰ47合成抑制剤

(57)【要約】
【課題】　コラーゲン合成を抑制することによって、細胞外マトリックス産生の亢進の病態を示す病気を治療することができる、分子量４７キログルトンの熱ショックタンパク質の合成抑制剤を提供する。
【解決手段】　ジンセノサイド類を有効成分として含有する。

【特許請求の範囲】
【請求項１】　ジンセノサイド類を有効成分として含有することを特徴とする、分子量４７キロダルトンの熱ショックタンパク質の合成抑制剤。
【請求項２】　ジンセノサイド類がジンセノサイドRg$_1$である、請求項１に記載の分子量４７キロダルトンの熱ショックタンパク質の合成抑制剤。
【請求項３】　ジンセノサイド類を含有する植物の抽出物を有効成分として含有することを特徴とする、分子量４７キロダルトンの熱ショックタンパク質の合成抑制剤。
【請求項４】　ニンジン又はコウジンの抽出物を有効成分として含有することを特徴とする、分子量４７キロダルトンの熱ショックタンパク質の合成抑制剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は、ジンセノサイド類、特にジンセノサイドRg$_1$を有効成分として含有する、分子量が４７キログルトン（ｋＤ）の熱ショックタンパク質（以下、ＨＳＰ４７と称する）の合成抑制剤に関する。本発明のＨＳＰ４７合成抑制剤は、特に、臓器内のコラーゲンの合成を抑制することにより肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス（細胞外基質）産生亢進の病態を示す病気の患者の生理学的状態を有効に改善させ、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気を効果的に治療することができる。
【０００２】
【従来の技術】近年、コラーゲンなどの細胞外マトリックスの産生の亢進の病態を示す病気が大きな問題となっている。ここでいう細胞外マトリックス産生の亢進の病態を示す病気とは、例えば肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどを含む。
【０００３】例えば、死亡者がわが国だけでも年間約２万人にものぼるといわれている肝硬変は、肝臓が結合組織の増殖のため固くなる病気の総称で、種々の慢性肝疾患の終末像であるといわれ、肝全体にわたるびまん性の肝線維症である。すなわち、炎症などの肝傷害が長期に及ぶ慢性肝炎においては、線維芽細胞や伊東細胞などの細胞外マトリックス（特にＩ型コラーゲン）産生の著しい亢進を伴い肝臓は線維化する。肝の線維化が慢性的に進行すると、ますます正常な肝再生は妨害され、肝細胞に置き換わり、線維芽細胞とＩ型コラーゲンを主体とする細胞外マトリックスが肝組織のかなりの部分を占め、多くの偽小葉からなる肝硬変に至る。肝硬変の進行に伴い、線維隔壁が肝全体に進展し、その結果生じる血流の異常は、肝実質細胞の変性を更に押し進める一因にもなり、肝硬変における悪循環が続くことになり、更にはアルコール、ウイルス、自己免疫等種々の原因によって、肝臓中に多量の膠質線維が生成され、肝細胞の壊死と機能消失とが生じ、肝硬変患者は遂には死に至る。Ｉ型コラーゲンは正常肝では全タンパク質量の約２％を占めるが、肝硬変となると１０～３０％を占めるようになる。
【０００４】また、間質性肺疾患は、肺胞及び肺胞管のみならず、しばしば呼吸細気管支や終末気管支も巻き込む下部気道の慢性炎症（肺胞炎 alveolitis)とその結果である間質の線維化と肺胞内線維化を特徴とする疾患群である。ここでいう間質性肺疾患とは、例えば、間質性肺炎、肺線維症などのびまん性間質性肺疾患、特発性肺線維症、透過性肺水腫、膠原病肺、サルコイドーシスなどを含む。間質性肺疾患においては、線維化組織では細胞外マトリックスの過剰な産生と蓄積が認められている。すなわち、間質性肺疾患の肺線維化組織では、肥大した間質に著明なＩ型及びIII型コラーゲンの集積がみられており、特にIII型コラーゲンは、線維化の早期に肥厚した肺胞中隔に集積し、病期が進行し、後期にはＩ型コラーゲンが増加し、主要なコラーゲンとなる。基底膜は早期に破壊されており、肺胞腔側へのコラーゲン線維の侵入が観察される。
【０００５】また、慢性腎不全とは慢性腎炎症候群の結果、腎機能の荒廃により体内の恒常性が維持できなくなった状態である。慢性腎不全の進行を病理学的にみると糸球体硬化と間質線維化の進行である。糸球体硬化症は、メサンギウム領域を中心とした細胞外マトリックスの増生である。メサンギウム硬化症の成分は正常と比較し、著明にIV型コラーゲンなどの糸球体基底膜の成分が増加し、また間質成分であるＩ型コラーゲンも硬化症部位に一致して増生している。すなわち、慢性に経過する糸球体硬化に対しては、細胞外マトリックスの産生亢進が大きな要因である。ここで慢性腎不全に陥いる疾患とは、例えばＩｇＡ腎症、巣状糸球体硬化症、膜性増殖性腎炎、糖尿病性腎症、慢性間質性腎炎、慢性糸球体腎炎などを含む。
【０００６】また、心筋細胞は高度に分化した細胞で、分裂して増殖する能力を持ち合わせていない。したがって、心臓に何らかの負荷が加わると、心筋細胞はその一つ一つが肥大して収縮力を増大させ、心機能を保とうとする。更に、負荷が長時間持続すると、虚血の要因を中心に多彩な障害が蓄積され、負荷に対する代償機構に破綻をきたし、心筋の収縮力は急激に低下し、心臓のポンプ機能は損なわれて、心不全に陥いることが知られてお

り、心肥大は我が国における心不全の成因として最も大きな部分を占めている。また、心肥大の形成は、心不全発症の最大の危険因子になるばかりでなく、虚血性心疾患や重篤な心室性不整脈の合併率が有意に高くなり、生命予後を独立に規定する要因になっている。心肥大進展時には個々の心筋細胞が著しく肥大するだけでなく、その心筋細胞をしっかり束ねるために、間質の線維化が促進され、細胞外マトリックスであるコラーゲンが増加する。また、心筋炎・心筋虚血などにより心筋細胞を失うと、コラーゲンが生合成され間隙を置換する。間質の線維化が過剰に進展すると、その結果、心筋は固くなり、拡張が障害される。更に、筋小体機能も低下して心筋の拡張期の弛緩も障害される。その他、術後の瘢痕や熱傷性瘢痕、あるいは強皮症、動脈硬化等の細胞外マトリックス産生亢進の病態を示す病気は、何らかの原因によりコラーゲン合成の異常亢進が起こり、線維化が進んで組織の硬化変化を生ずることが主要な成因と考えられている。

【０００７】また、血管新生においても基底膜及び基底膜中のコラーゲン合成が、重要な役割をはたすことが指摘されている（Maragoudakis, E., Sarmonika, M., and Panoutsacopoulous, M., "J. Pharmacol. Exp. Ther.", 244 : 729, 1988 ; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119 : 1768, 1986）。血管新生による疾患としては、例えば、糖尿病性網膜症、後水晶体線維増殖症、角膜移植に伴う血管新生、緑内症、眼腫瘍、トラコーマ、幹せん、化膿性肉芽腫、血管腫、線維性血管腫、肥大性はん痕、肉芽、リューマチ性関節炎、浮腫性硬化症、アテローム性動脈硬化症、各種腫瘍などが知られている。このようにコラーゲンなどの細胞外マトリックスの産生の亢進の病態を示す病気が大きな問題となっているにもかかわらず、従来では副作用や薬理効果等の種々の面で満足すべき細胞外マトリックス合成抑制剤（例えば、コラーゲン合成抑制剤）は未だ開発されていなかったのである。

【０００８】一方、熱ショックタンパク質（heat shock protein；ＨＳＰ、ストレスタンパク質ともいう）は、細胞を何らかのストレス、例えば熱、重金属、薬剤、アミノ酸類似体、又は低酸素（低濃度酸素）などで刺激することにより、細胞に発現される一群のタンパク質である。熱ショックタンパク質は、自然界に普遍的に存在しており、細菌、酵母、植物、昆虫、及びヒトを含む高等動物により産生される。ＨＳＰは、その種類は多種多様であるが、分子量の大きさからＨＳＰ９０ファミリー（例えば、９０ｋＤ又は１１０ｋＤのＨＳＰなど）、ＨＳＰ７０ファミリー（例えば、７０～７３ｋＤのＨＳＰなど）、ＨＳＰ６０ファミリー（例えば、５７～６８ｋＤのＨＳＰなど）、低分子ＨＳＰファミリー（例えば、２０ｋＤ、２５～２８ｋＤ、又は４７ｋＤのＨＳＰなど）の４ファミリーに大別することができる。なお、本明細書においては、特定分子量を有するＨＳＰを、ＨＳＰとその直後に記載する数字とによって示すものとし、例えば、分子量４７ｋＤのＨＳＰを『ＨＳＰ４７』と称するものとする。以上のように、ＨＳＰには多くの種類が存在するが、これらは分子量だけでなく、構造、機能、又は性質などもそれぞれ異なるものである。ストレスへの応答に加えて、これらのタンパク質の中には構成的に合成されるものがあり、正常な環境の下で、タンパク質のフォールディング、アンフォールディング、タンパク質サブユニットの会合、タンパク質の膜輸送のような、必須の生理的な役割を演じていることが示されている。熱ショックタンパク質としてのこれらの機能は、分子シャペロンと称される。

【０００９】ＨＳＰ４７は、永田等によって１９８６年に発見されたタンパク質で、分子量４７キロダルトンの塩基性タンパク質（ｐＩ＝９．０）である。ＨＳＰ４７の発現が増大するにつれて、コラーゲンの合成も増加することが様々な細胞で示されている（"J. Biol. Chem.", 261 : 7551, 1986 ; "Eur. J. Biochem.", 206 : 323, 1992 ; "J. Biol. Chem.", 265 : 992, 1990 ; "J. Clin. Invest.", 94 : 2481, 1994）。すなわち、ＨＳＰ４７は、細胞内で小胞体内でのプロコラーゲンのプロセシング、三重鎖ヘリックス形成、あるいは小胞体からゴルジ装置へのプロコラーゲン輸送・分泌という局面で、コラーゲンの特異的分子シャペロンとして機能しているとされているので、増大したＨＳＰ４７発現は、細胞外マトリックスにおけるコラーゲン分子の蓄積を刺激する。このようにコラーゲン結合熱ショックタンパク質であるＨＳＰ４７は、発現と同様に機能においても、細胞外マトリックスタンパク質であるコラーゲンに密接に関連した熱ショックタンパク質である。

【００１０】

【発明が解決しようとする課題】本発明者らは、上記事情に鑑み、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気の患者の生理学的状態を有効に改善させ、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気を効果的に治療することのできる、細胞外マトリックス合成抑制剤を提供するために、種々検討を重ねてきた。

【００１１】上記したように、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの線維症は臓器内の細胞外マトリックスの著しく

増加した病態が主病変と理解されている。肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生亢進の病態を示す病気に伴う線維化は、コラーゲン生合成増加やコラーゲン分解能の低下により生ずると考えられている。例えば、肝の線維化において、I型、III型、IV型コラーゲンの合成活性化が起こるが、特に主要成分であるI型コラーゲンの合成活性化が重要な意味をもつ。

【0012】こうした状況下で、本発明者らは、意外にも、ニンジン又はコウジン等の成分であるジンセノサイド類、特にジンセノサイドRg_1が、病態を示す組織の細胞におけるHSP47の合成を特異的に抑制することを見出した。すなわち、ジンセノサイド類を投与することにより、細胞内でのHSP47の合成を抑制し、臓器内でのコラーゲン合成を抑制し、ひいては肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気の治療が可能であることを見出したのである。本発明はこうした知見に基づくものであり、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気を効果的に治療することのできるHSP47合成抑制剤であって、細胞内でのコラーゲンの成熟及び輸送過程に重要な役割を果たしているコラーゲン特異的な分子シャペロンであるHSP47の合成抑制剤を提供することを目的とする。

【0013】

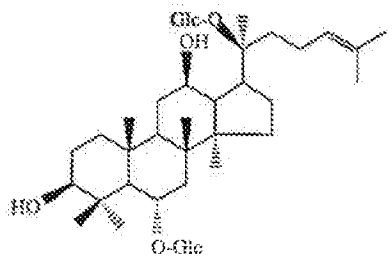
【課題を解決するための手段】従って、本発明は、ジンセノサイド類、特にジンセノサイドRg_1を有効成分として含有することを特徴とする、HSP47合成抑制剤に関する。

【0014】

【発明の実施の形態】以下、本発明について詳細に説明する。本発明の合成抑制剤は、有効成分としてジンセノサイド類を含有する。本明細書においてジンセノサイド類（ギンセノシド類；ginsenosides）とは、例えば、ジンセノサイドRo（ギンセノシドRo；ginsenoside Ro；チクセツサポニンV；chikusetsusaponinV；サポニンA；saponin A）、ジンセノサイドRa_1（ギンセノシドRa_1；ginsenoside Ra_1）、ジンセノサイドRa_2（ギンセノシドRa_2；ginsenoside Ra_2）、ジンセノサイドRb_1（ギンセノシドRb_1；ginsenoside Rb_1；サポニンD；saponin D）、ジンセノサイドRb_2（ギンセノシドRb_2；ginsenoside Rb_2）、ジンセノサイドRb_3（ギンセノシドRb_3；ginsenoside Rb_3）、ジンセノサイドRc（ギンセノシドRc；ginsenoside Rc）、ジンセノサイドRd（ギンセノシドRd；ginsenoside Rd）、ジンセノサイドRe（ギンセノシドRe；ginsenoside Re）、ジンセノサイドRf（ギンセノシドRf；ginsenoside Rf）、ジンセノサイドRg_1（ギンセノシドRg_1；ginsenoside Rg_1）、ジンセノサイドRg_2（ギンセノシドRg_2；ginsenoside Rg_2；チクセツサポニンI；chikusetsusaponinI）、ジンセノサイドRg_3（ギンセノシドRg_3；ginsenosideRg_3）、ジンセノサイドRh_1（ギンセノシドRh_1；ginsenoside Rh_1）、ジンセノサイドRh_2（ギンセノシドRh_2；ginsenoside Rh_2）、20-グルコジンセノサイドRf（20-グルコギンセノシドRf；20-glucoginsenoside Rf）、マロニルジンセノサイドRb_1（マロニルギンセノシドRb_1；maronylginsenoside Rb_1）、マロニルジンセノサイドRb_2（マロニルギンセノシドRb_2；maronylginsenoside Rb_2）、マロニルジンセノサイドRc（マロニルギンセノシドRc；maronylginsenosideRc）、マロニルジンセノサイドRd（マロニルギンセノシドRd；maronylginsenoside Rd）、チクセツサポニンIa（chikusetsusaponin Ia）、チクセツサポニンIb（chikusetsusaponin Ib）、チクセツサポニンIII（chikusetsusaponin III）、チクセツサポニンIV（chikusetsusaponinIV；サポニンB；saponin B）、チクセツサポニンIVa（chikusetsusaponin IVa；サポニンC；saponin C）、プロトパナキサジオール（protopanaxadiol）、プロトパナキサトリオール（protopanaxatriol）、オレアノール酸（oleanolic acid）等、又はこれらの化合物の立体異性体を意味する。本発明においては、それらのジンセノサイド類は、単独で用いることもできるし、あるいは、異なる複数のジンセノサイド類を組み合わせて同時に用いることもできる。

【0015】本発明の合成抑制剤において有効成分として使用することのできるジンセノサイド類としては、特に、ジンセノサイドRg_1が最も好適である。ジンセノサイドRg_1（ギンセノシドRg_1；ginsenoside Rg_1）は、式（I）：

【化1】



(I)

（式中、Ｇｌｃはβ－Ｄ－グルコピラノシル基である）で表され、分子式$C_{42}H_{72}O_{14}$及び分子量８０１．０３の化合物であり、例えば、ニンジン又はコウジン等の生薬に含まれている。

【００１６】本発明の合成抑制剤に含有されるジンセノサイド類は、化学合成によって、又は天然物から抽出して精製することによって、調製することができる。あるいは、市販品を用いてもよい。本発明の合成抑制剤において有効成分として用いるジンセノサイド類を、天然物から抽出する場合には、例えば、ジンセノサイド類を含有する植物の全体又は一部分（例えば、全草、葉、根、根茎、茎、根皮、若しくは花）をそのまま用いて、又は簡単に加工処理（例えば、乾燥、切断、湯通し、蒸気加熱、若しくは粉末化）したもの（例えば、生薬）を用いて抽出する。抽出条件は一般的に植物抽出に用いられる条件ならば特に制限はない。ジンセノサイド類を含有する植物としては、これに限定するものではないが、例えば、ウコギ科（Ａｒａｌｉａｃｅａｅ）のオタネニンジン（Ｐａｎａｘ ｇｉｎｓｅｎｇ Ｃ．Ａ．Ｍｅｙｅｒ；Ｐａｎａｘ ｓｃｈｉｎｓｅｎｇ Ｎｅｅｓ）、トチバニンジン〔Ｐａｎａｘｊａｐｏｎｉｃｕｓ Ｃ．Ａ．Ｍｅｙｅｒ；Ｐａｎａｘ ｓｃｈｉｎｓｅｎｇＮｅｅｓ ｖａｒ．ｊａｐｏｎｉｃｕｍ Ｍａｋｉｎｏ；Ｐａｎａｘ ｐｓｅｕｄｏ－ｇｉｎｓｅｎｇ（Ｗｉｌｌ．）ｓｕｂｓｐ．ｊａｐｏｎｉｃｕｓ Ｈａｒａ〕、サンシチニンジン〔Ｐａｎａｘ ｎｏｔｏｇｉｎｓｅｎｇ（Ｂｕｒｋｉｌｌ）Ｆ．Ｈ．Ｃｈｅｎ；Ｐａｎａｘ ｓａｎｃｈｉ Ｈｏｏ；Ｐａｎａｘｐｓｅｕｄｏ－ｇｉｎｓｅｎｇ Ｗａｌｌｉｃｈ ｖａｒ．ｎｏｔｏｇｉｎｓｅｎｇ（Ｂｕｒｋｉｌｌ）Ｈｏｏ ｅｔ Ｔｓｅｎｇ〕、又はアメリカニンジン（Ｐａｎａｘ ｑｕｉｎｑｕｅｆｏｌｉｕｍ Ｌ．）等を使用することができる。

【００１７】本発明におけるジンセノサイド類を生薬から抽出する場合、これに限定するものではないが、例えば、ニンジン又はコウジンから抽出することが好ましい。ニンジン（人参；Ｇｉｎｓｅｎｇ Ｒａｄｉｘ）とは、オタネニンジンの細根を除いた根又はこれを軽く湯通ししたものを意味し、それらの部分を単独であるいは任意に組み合わせて使用することができる。また、コウジン（紅参；Ｒｅｄ Ｇｉｎｓｅｎｇ；Ｇｉｎｓｅｎｇ Ｒａｄｉｘ Ｒｕｂｒａ）とは、オタネニンジンの根を蒸したものを意味し、それらの部分を単独であるいは任意に組み合わせて使用することができる。

【００１８】本発明による合成抑制剤において有効成分として用いることのできるニンジン抽出物又はコウジン抽出物は、前記のジンセノサイド類、特にジンセノサイドＲｇ$_1$を含有していればよく、従って、ニンジン又はコウジンの粗抽出物を用いることができる。本発明で用いることのできるニンジン抽出物又はコウジン抽出物の製造方法としては、ニンジン又はコウジンを、水（例えば、冷水、温水、好ましくは熱湯）によって抽出するか、又は有機溶媒を用いて抽出することによって、得ることができる。有機溶媒としては、例えば、炭素数１～６のアルコール（例えば、メチルアルコール、エチルアルコール、ｎ－プロピルアルコール、イソプロピルアルコール、若しくはブチルアルコール）、エステル（例えば、酢酸メチル、酢酸エチル、酢酸プロピル、若しくは酢酸ブチル）、ケトン（例えば、アセトン若しくはメチルイソブチルケトン）、エーテル、石油エーテル、ｎ－ヘキサン、シクロヘキサン、トルエン、ベンゼン、炭化水素のハロゲン誘導体（例えば、四塩化炭素、ジクロロメタン、若しくはクロロホルム）、ピリジン、グリコール（例えば、プロピレングリコール、若しくはブチレングリコール）、ポリエチレングリコール、又はアセトニトリルなどを用いることができ、これらの有機溶媒を単独、又は適宜組み合わせ、一定の比率で混合し、更には無水又は含水状態で用いることができる。好ましくは、メチルアルコール等が望ましい。水抽出又は有機溶媒抽出の方法としては、通常の生薬抽出に用いられる方法を用いることができ、例えば、（乾燥）ニンジン又はコウジン１重量部に対し、水又は有機溶媒３～３００重量部を用いて、撹拌しながら、その沸点以下の温度で加熱還流、常温で超音波抽出、あるいは冷浸することが望ましい。抽出工程は、通常は５分～７日間、好ましくは１０分～２４時間実施し、必要に応じて、撹拌等の補助的手段を加えることにより、抽出時間を短縮することができる。

【００１９】抽出工程終了後、沪過又は遠心分離等の適当な方法により、水又は有機溶媒抽出液から、不溶物を分離して粗抽出物を得ることができる。なお、本発明の合成抑制剤において、天然物より抽出、分画したジンセノサイド類、特にジンセノサイドＲｇ$_1$を用いる場合には、前記の粗抽出物を特に精製することなく、そのまま使用してもよい。常法による水抽出物又は有機溶媒抽出物の他に、前記の粗抽出物を各種有機溶媒又は吸着剤等により、更に処理した精製抽出物も、本発明の合成抑制剤の有効成分として用いることができる。これらの粗抽出物及び各種の精製処理を終えた精製抽出物を含むニンジン抽出物又はコウジン抽出物は、抽出したままの溶液を用いても、溶媒を濃縮したエキスを用いても良いし、溶媒を留去し乾燥した粉末、更には結晶化して精製した

もの、あるいは粘性のある物質を用いても良く、またそれらの希釈液を用いることもできる。こうして得られたニンジン抽出物又はコウジン抽出物は、ニンジン又はコウジンに含まれるジンセノサイド類を混合物として含み、同時に原料のニンジン又はコウジンに由来する不純物を含んでいる。

【００２０】本発明の合成抑制剤は、ジンセノサイド類、又はジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特には、ニンジン抽出物又はコウジン抽出物）を、それ単独で、又は好ましくは製剤学的若しくは獣医学的に許容することのできる通常の担体と共に、動物、好ましくは哺乳動物（特にはヒト）に投与することができる。投与剤型としては、特に限定がなく、例えば、散剤、細粒剤、顆粒剤、錠剤、カプセル剤、懸濁液、エマルジョン剤、シロップ剤、エキス剤、若しくは丸剤等の経口剤、又は注射剤、外用液剤、軟膏剤、坐剤、局所投与のクリーム、若しくは点眼薬などの非経口剤を挙げることができる。これらの経口剤は、例えば、ゼラチン、アルギン酸ナトリウム、澱粉、コーンスターチ、白糖、乳糖、ぶどう糖、マンニット、カルボキシメチルセルロース、デキストリン、ポリビニルピロリドン、結晶セルロース、大豆レシチン、ショ糖、脂肪酸エステル、タルク、ステアリン酸マグネシウム、ポリエチレングリコール、ケイ酸マグネシウム、無水ケイ酸、又は合成ケイ酸アルミニウムなどの賦形剤、結合剤、崩壊剤、界面活性剤、滑沢剤、流動性促進剤、希釈剤、保存剤、着色剤、香料、矯味剤、安定化剤、保湿剤、防腐剤、又は酸化防止剤等を用いて、常法に従って製造することができる。例えば、１重量部のジンセノサイドＲｇ$_1$と９９重量部の乳糖とを混合して充填したカプセル剤などである。

【００２１】非経口投与方法としては、注射（皮下、静脈内等）、又は直腸投与等が例示される。これらのなかで、注射剤が最も好適に用いられる。例えば、注射剤の調製においては、有効成分としてのジンセノサイド類（特にはジンセノサイドＲｇ$_1$）、又はジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特には、ニンジン抽出物又はコウジン抽出物）の他に、例えば、生理食塩水若しくはリンゲル液等の水溶性溶剤、植物油若しくは脂肪酸エステル等の非水溶性溶剤、ブドウ糖若しくは塩化ナトリウム等の等張化剤、溶解補助剤、安定化剤、防腐剤、懸濁化剤、又は乳化剤等を任意に用いることができる。また、本発明の合成抑制剤は、徐放性ポリマーなどを用いた徐放性製剤の手法を用いて投与してもよい。例えば、本発明の合成抑制剤をエチレンビニル酢酸ポリマーのペレットに取り込ませて、このペレットを治療すべき組織中に外科的に移植することができる。

【００２２】本発明の合成抑制剤は、これに限定されるものではないが、ジンセノサイド類を、０．０１～９９重量％、好ましくは０．１～８０重量％の量で含有することができる。また、ジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特には、ニンジン抽出物又はコウジン抽出物）を有効成分として含有する本発明の合成抑制剤は、その中に含まれるジンセノサイド類（特にはジンセノサイドＲｇ$_1$）が前記の量範囲になるように適宜調整して、調製することができる。なお、ジンセノサイド類を含有する植物の抽出物、例えば、ジンセノサイド類を含有する生薬の抽出物（特には、ニンジン抽出物又はコウジン抽出物）を有効成分として含有する合成抑制剤を、経口投与用製剤とする場合には、製剤学的に許容することのできる担体を用いて、製剤化することが好ましい。

【００２３】本発明の合成抑制剤を用いる場合の投与量は、病気の種類、患者の年齢、性別、体重、症状の程度、又は投与方法などにより異なり、特に制限はないが、ジンセノサイドＲｇ$_1$量として通常成人１人当り１ｍｇ～１０ｇ程度を、１日１～４回程度にわけて、経口的に又は非経口的に投与する。更に、用途も医薬品に限定されるものではなく、種々の用途、例えば、機能性食品や健康食品として飲食物の形で与えることも可能である。

【００２４】

【作用】上記したように、本発明の合成抑制剤に含有されるジンセノサイド類、特にジンセノサイドＲｇ$_1$は、細胞内のＨＳＰ４７合成を特異的に抑制する作用があるので、前記ジンセノサイド類を投与すると細胞内でのＨＳＰ４７生合成が特異的に減少し、コラーゲンの生合成が抑制される。その結果、細胞外マトリックス産生も抑制されることになる。従って、前記ジンセノサイド類は、コラーゲンの増加を伴う細胞外マトリックス産生亢進の病態を示す病気、例えば肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの予防及び治療に使用することができる。すなわち、本発明の合成抑制剤は、コラーゲン特異的シャペロンであるＨＳＰ４７の合成を抑制することによりコラーゲンの合成を抑制する。

【００２５】また、前記のように、血管新生においても、基底膜及び基底膜中のコラーゲン合成が重要な役割をはたすことが指摘されているので、本発明の合成抑制剤は、血管新生の異常増殖に基づく多くの疾患の予防治療薬として極めて有用であり、先に述べたような各疾患、すなわち糖尿病性網膜症、後水晶体線維増殖症、角膜移植に伴う血管新生、緑内症、眼腫瘍、トラコーマ、乾せん、化膿性肉芽腫、血管腫、線維性血管腫、肥大性はん痕、肉芽、リューマチ性関節炎、浮腫性硬化症、アテローム性動脈硬化症及び各種腫瘍などに用いることができる。更に、Ｉ型コラーゲンとフィブロネクチンを基

本骨格とする間質（interstitial stroma)が癌の転移において、離脱した癌細胞が近傍の脈管に侵入するまでのガイド役を果たすことが、明らかとなっているので〔"BIOTHERAPY", 7 (8) : 1181, 1993〕、本発明の合成抑制剤を投与することにより、癌の転移を抑制することも可能である。

【００２６】

【実施例】以下、実施例によって本発明を具体的に説明するが、これらは本発明の範囲を限定するものではない。

実施例１：抗ＨＳＰ４７ポリクローナル抗体の作製

（１）抗ＨＳＰ４７ポリクローナル抗体の調製

ヒトＨＳＰ４７のＮ末端から２～１６番目のアミノ酸配列に対応するアミノ酸１５個からなるペプチド〔以下、ヒトＨＳＰ４７ペプチド（２－１６）と称する〕を自動ペプチド合成装置（ＰＳＳＭ－８システム、島津製作所）を用いて作製し、スクシニミジル４－（ｐ－マレイミドフェニル）ブチレート〔ＳＭＰＢ：Succinimidyl 4-(p-maleimidophenyl)butyrate〕を架橋剤として用い、常法（"Biochemistry", 13 : 690, 1979)によりラクトグロブリンと結合させ、感作抗原を作製した。この感作抗原１５０μｇを含むリン酸緩衝生理食塩水〔組成：ＫＣｌ＝０．２ｇ／ｌ，KH_2PO_4＝０．２ｇ／ｌ，ＮａＣｌ＝８ｇ／ｌ，Na_2HPO_4（無水）＝１．１５ｇ／ｌ：以下ＰＢＳ（－）と称する：コスモバイオ，カタログ番号320-01〕０．２ｍｌと、等量のフロイント完全アジュバント（ヤトロン，カタログ番号RM606-1)とを混和し、得られた混合液０．２ｍｌを、ルーラット（６週齢，雌性：日本クレア）の皮下に投与し、免疫した。同様の方法で第２次及び第３次免疫を繰り返した後、アジュバント（Hunter's TiterMax ; CytRx Corporation,米国ジョージア州）を用いて６回免疫感作を行った。感作動物より採血し、常法により血清を分離して採取し、以下に示す酵素抗体法（ＥＬＩＳＡ法）及びウェスタンブロット法によって血清中の抗体価を測定した。

【００２７】（２）酵素抗体法（ＥＬＩＳＡ法）による抗ＨＳＰ４７ポリクローナル抗体特性の評価

前項（１）で調製したヒトＨＳＰ４７ペプチド（２－１６）をＰＢＳ（－）に溶解し、１０μｇ／ｍｌの濃度のペプチド溶液を調製し、リジットアセイプレート（ファルコン，カタログ番号3910）の各ウェルに前記ペプチド溶液を５０μｌずつ滴下した。最も外側のウェルにはＰＢＳ（－）５０μｌのみを入れ、湿潤下で４℃にて一晩放置した後、前記ペプチド溶液を捨て、ＰＢＳ（－）を用いて各ウェルを洗浄した後、１％ウシ血清アルブミン（以下、ＢＳＡと略称する）を含むＰＢＳ（－）１００μｌを各ウェルに入れ、室温下で１時間放置した。ＰＢＳ（－）で３回洗浄した後、前項（１）で取得したルーラット血清５０μｌを各ウェルに入れ、１時間室温にて放置した。ＰＢＳ（－）で３回洗浄した後、各ウェルに２次抗体としてペルオキシダーゼ標識抗ラットＩｇＧ５０μｌを入れ、室温下で１時間放置した。ＰＢＳ（－）で３回洗浄した後、過酸化水素水４μｌを加えた０．１Ｍクエン酸バッファー（ｐＨ４．５）１０ｍｌにｏ－フェニレンジアミン（ＯＰＤ）タブレット（シグマ，カタログ番号P8287）１個（１０ｍｇ）を溶解して調製した基質液１００μｌずつを各ウェルに滴下し、室温にて遮光下で３０分間放置した後、各ウェルの４９２ｎｍの吸光度をマイクロプレートリーダー（東ソー，ＭＰＲ－Ａ４ｉ型）にて測定した。抗体価の上昇が確認された血清を抗ヒトＨＳＰ４７ポリクローナル抗体として以下の実施例に用いた。

【００２８】（３）ウェスタンブロット法による抗ＨＳＰ４７ポリクローナル抗体特性の評価

Ｌａｅｍｍｌｉのバッファー系（Laemmli, N. K., "Nature", 283 : pp. 249-256, 1970)を用いて、ＨｅＬａ細胞のライセートのドデシル硫酸ナトリウム（ＳＤＳ）ポリアクリルアミドゲル電気泳動を、以下の方法に従って行った。濃縮ゲルの調製は次のように行った。蒸留水６．１ｍｌ、０．５Ｍトリス（バイオ・ラッド，カタログ番号161-0716）－ＨＣｌ（ｐＨ６．８）２．５ｍｌ、１０％ＳＤＳ（バイオ・ラッド，カタログ番号161-0301）１００μｌ、及び３０％アクリルアミド（バイオ・ラッド，カタログ番号161-0101）／Ｎ，Ｎ’－メチレンビスアクリルアミド（バイオ・ラッド，カタログ番号161-0201）１．３ｍｌを混合して、１５分間脱気し、１０％過硫酸アンモニウム（バイオ・ラッド，カタログ番号161-0700）５０μｌ及びＮ，Ｎ，Ｎ’，Ｎ’－テトラメチルエチレンジアミン（以下、ＴＥＭＥＤと略称する）（バイオ・ラッド，カタログ番号161-0800）１０μｌを加えて、濃縮ゲルを調製した。また、分離ゲルの調製は次のように行った。蒸留水４．０４５ｍｌ、１．５Ｍトリス－ＨＣｌ（ｐＨ８．８）２．５ｍｌ、１０％ＳＤＳ１００μｌ、及び３０％アクリルアミド／Ｎ，Ｎ’－メチレンビスアクリルアミド３．３ｍｌをゆっくり混合して、１５分間アスピレータで脱気し、１０％過硫酸アンモニウム５０μｌ、及びＴＥＭＥＤ５μｌを加えた。泳動バッファーとしては、トリス９．０ｇ、グリシン（バイオ・ラッド，カタログ番号161-0717）４３．２ｇ、及びＳＤＳ３．０ｇに蒸留水を加えて６００ｍｌにし、これを蒸留水で５倍希釈したものを用いた。サンプルバッファーは、蒸留水２ｍｌ、２Ｍトリス－ＨＣｌ（ｐＨ６．８）５００μｌ、ＳＤＳ０．３２ｇ、β－メルカプトエタノール８００μｌ、及び０．０５％（ｗ／ｖ）ブロモフェノールブルー（バイオ・ラッド，カタログ番号161-0404）４００μｌを混合したものを用いた。

【００２９】５％二酸化炭素条件下で、３７℃で、１０％非働化ウシ胎児血清（以下、ＦＢＳと略称する）を含むＭＥＭ培地中でＨｅＬａ細胞を培養し、そのライセートを調製した。得られたＨｅＬａ細胞ライセートのＳＤ

Ｓ－ポリアクリルアミドゲル電気泳動を行った後、０．４５μｍニトロセルロース膜（Schleicher & Schuell，カタログ番号401196）にゲルを密着させ、タンパク質転写装置（Trans-Blot Electrophoretic Transfer Cell：バイオ・ラッド）を用いて、室温にて１００Ｖで、３時間ブロッティングを行った。ブロッティングバッファーとしては０．０２５Ｍトリス及び０．１９２ＭグリシンよりなりｐＨ８．５に調整されたトリスグリシンバッファー（Tris Gly Running and Blotting Buffer；Emprotech,米国マサチューセッツ州，カタログ番号SA100034）にメチルアルコールを２０％になるように加えて調製したバッファーを用いた。ブロッティング後、５％スキムミルク（雪印乳業）を含むＰＢＳ（－）溶液にニトロセルロース膜を室温にて３０分間浸し、ブロッキングを行った。ブロッキング後、スクリーナーブロッター（サンプラテック）を用いて、前項（１）で取得したルーラット血清を１次抗体として、１次抗体反応を行った。１次抗体反応は、２％スキムミルク（雪印乳業）を含むＰＢＳ（－）にて１０倍希釈した前記ルーラット血清２００μｌで、室温にて１２０分間行った。１次抗体反応終了後、スロー・ロッキング・シェイカーを用いて、ＰＢＳ（－）で5分間の振盪を２回、０．１％Ｔｗｅｅｎ２０（バイオ・ラッド，カタログ番号170-6531）を含むＰＢＳ（－）溶液で１５分間の振盪を４回、更にＰＢＳ（－）で5分間の振盪を２回行うことにより、ニトロセルロース膜を洗浄した。洗浄終了後、ペルオキシダーゼ標識ヤギ抗ラットＩｇＧ抗体（Southern Biotechnology，カタログ番号3030-05)を、２％スキムミルクを含むＰＢＳ（－）溶液で５０００倍に希釈した溶液５ｍｌを用いて、２次抗体反応を２時間行った。反応終了後、ＰＢＳ（－）溶液、及び０．１％Ｔｗｅｅｎ２０を含むＰＢＳ（－）溶液で、１次抗体反応後の洗浄と同じ条件下にてニトロセルロース膜の洗浄を行った。

【００３０】余分なＰＢＳ（－）溶液を除去した後、ウェスタンブロッティング検出試薬（ECL Western blotting detection reagent；アマーシャム，カタログ番号RPN2106)をニトロセルロース膜上に振りかけ、１分間室温にて静置した後、余分な検出試薬を除去し、ニトロセルロース膜をラップに包み、反応面をＸ線フィルム（コダック X-OMAT、AR,カタログ番号165 1454）に密着させて露光させた。現像後、ＨＳＰ４７に相当する分子量４７キログルトン付近のバンドを測定することによって、抗ＨＳＰ４７ポリクローナル抗体の反応性の検討を行った。抗体価の上昇が確認された血清を、抗ヒトＨＳＰ４７ポリクローナル抗体として、以下の実施例に用いた。

【００３１】実施例２：ヒト培養癌細胞のＨＳＰ発現量の測定

（１）ヒト培養癌細胞の培養

ヒト乳癌細胞株ＭＣＦ７（ＡＴＣＣ　ＨＴＢ　２２）を、10^{-8}Ｍβ－エストラジオール及び１０％非働化ＦＢＳを含むＲＰＭＩ１６４０培地中で、５％二酸化炭素条件下で、熱ショック処理時以外は、３７℃で培養した。

【００３２】（２）ジンセノサイドＲｇ$_1$処理及び熱ショック処理

播種２日後の前記ヒト乳癌細胞株ＭＣＦ７の培地中に、最終濃度１００μＭになるように前記式（Ｉ）で表されるジンセノサイドＲｇ$_1$（松浦薬業）を添加し、２４時間培養した。その後、４５℃にて１５分間熱ショック処理をしてから、３７℃にて終夜培養した。対照試験は、ジンセノサイドＲｇ$_1$を添加しないこと以外は前記と同様に実施した。

【００３３】（３）ヒト培養癌細胞でのＨＳＰ発現量の測定

前項（２）で処理した各細胞を、以下に示す方法によりホモジナイズし、ＨＳＰ発現量をウェスタンブロット法にて測定した。すなわち、前項（２）で処理した細胞をＰＢＳ（－）で洗浄した後、ライシスバッファー（ｌｙｓｉｓ　ｂｕｆｆｅｒ）〔１．０％ＮＰ－４０、０．１５Ｍ塩化ナトリウム、５０ｍＭトリス－ＨＣｌ（ｐＨ８．０）、５ｍＭ－ＥＤＴＡ、２ｍＭ－Ｎ－エチルマレイミド、２ｍＭフェニルメチルスルホニルフルオリド、２μｇ／ｍｌロイペプチン及び２μｇ／ｍｌペプスタチン〕１ｍｌを加え、氷上で２０分間静置した。その後、４℃で１２０００ｒｐｍにて、２０分間、遠心を行った。遠心後の上清１０μｌをＰＢＳ（－）７９０μｌに加え、更にプロテインアッセイ染色液（Dye Reagent Concentrate：バイオラッド，カタログ番号500-0006）２００μｌを加えた。５分間、室温にて静置した後、５９５ｎｍで吸光度を測定してタンパク質定量を行った。タンパク質定量を行った試料を用いて、Ｌａｅｍｍｌｉのバッファー系にて、等量のタンパク質を含むライセートのＳＤＳポリアクリルアミドゲル電気泳動を行った。電気泳動後、実施例１で述べた方法に従って、ブロッティング及びそれに続くブロッキングを行った。すなわち、タンパク質転写装置（Trans-Blot Electrophoretic Transfer Cell：バイオ・ラッド）を用いて、室温にて１００Ｖにて、０．４５μｍニトロセルロース膜（Schleicher & Schuell，カタログ番号401196）にゲルを密着させ、３時間ブロッティングを行った。ブロッティングバッファーとしては、前記実施例１（３）で用いたバッファーと同じものを用いた。ブロッティング後、ニトロセルロース膜を１０％スキムミルク（雪印乳業）－ＰＢＳ（－）溶液に室温にて３０分間、インキュベートし非特異的結合をブロックした。

【００３４】ブロッキング後、ニトロセルロース膜の上で、実施例１で製造した抗ヒトＨＳＰ４７ラットポリクローナル抗体により、１次抗体反応を行った。その後、ＰＢＳ（－）で5分間ずつ、溶液を取り替えて２回の洗浄をスロー・ロッキング・シェイカーによって行い、更

にＰＢＳ（－）－０．１％Ｔｗｅｅｎ２０（バイオ・ラッド，カタログ番号170-6531）溶液で１５分間ずつ，溶液を取り替えて４回の洗浄を行った。最終的に、ＰＢＳ（－）で５分間ずつ、２回の洗浄を行った。洗浄終了後、ペルオキシダーゼ標識ヤギ抗ラットＩｇＧ抗体（Southern Biotechnology，カタログ番号3030-05)を、２％スキムミルクを含むＰＢＳ（－）溶液で５０００倍に希釈して調製した抗体溶液５ｍｌを用いて、２時間、２次抗体反応を行った。反応終了後、ニトロセルロース膜に関して、ＰＢＳ（－）溶液で５分間ずつ溶液を変えて２回、更にＰＢＳ（－）－０．１％Ｔｗｅｅｎ２０溶液で１５分間ずつ溶液を変えて５回の洗浄をスロー・ロッキング・シェイカーにより行った。最後にＰＢＳ（－）溶液で５分間ずつ２回の洗浄を行った。余分なＰＢＳ（－）溶液を除去した後、ウェスタンブロッティング検出試薬（ECL Westernblotting detection reagent；Amersham，カタログ番号RPN2106)をニトロセルロース膜上に振りかけ、１分間インキュベートした後、余分な検出試薬を除去し、ニトロセルロース膜をラップに包み、反応面をＸ線フィルム（コダック X-OMAT,AR,カタログ番号165 1454）に密着させて露光し、現像してＨＳＰ４７の有無の検討を行った。

【００３５】対照試験、すなわち、ジンセノサイドＲｇ$_1$を添加しなかった乳癌細胞株ＭＣＦ７では，分子量約４７ｋＤのバンドが一本検出された。ジンセノサイドＲｇ$_1$を添加した乳癌細胞株ＭＣＦ７では、相当するバンドが極めて薄いバンドであった。すなわち、ジンセノサイドＲｇ$_1$は、ＨＳＰ４７の発現を抑制する合成抑制剤の活性を有するものと結論することができ、この事実は、ジンセノサイドＲｇ$_1$が細胞外マトリックス産生の亢進に抑制的に働くことを示している。

【００３６】

【発明の効果】以上詳述したように、本発明の合成抑制剤は、例えば、肝硬変、間質性肺疾患、慢性腎不全（又は慢性腎不全に陥いる疾患）、心肥大、術後の瘢痕や熱傷性瘢痕、交通事故等の後に生じるケロイドや肥厚性瘢痕、強皮症、動脈硬化、又は関節リウマチなどの細胞外マトリックス産生の亢進の病態を示す病気に罹患した細胞にみられるコラーゲン合成亢進を改善する作用を有する。従って、本発明による合成抑制剤を投与することにより、臓器、組織の線維化、硬化が阻止され、その結果、前記病気の患者の生理学的状態を有効に改善させ、前記病気を効果的に治療することができる。また、本発明の合成抑制剤は、血管新生の異常増殖を伴う各種疾患の予防治療にも有用である。更に、Ｉ型コラーゲンとフィブロネクチンを基本骨格とする間質が、癌の転移において離脱した癌細胞が近傍の脈管に侵入するまでのガイド役を果たすことが、明らかとなっているので、本発明の合成抑制剤を投与することにより、癌の転移を抑制することも可能である。

フロントページの続き

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		ＡＤＡ				ＡＤＡＭ

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]The molecular weight in which this invention contains JINSENO sides, especially JINSENO side Rg_1 as an active principle is related with synthetic inhibitor of the heat shock protein (HSP47 is called hereafter) of 47 kilodalton (kD). Especially HSP47 synthetic inhibitor of this invention by controlling composition of collagen in an organ Liver cirrhosis, An interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, The keloid and the hypertrophic scar which are produced after a postoperative scar, a burn nature scar, a traffic accident, etc., The physiological status of the sick patient who shows the symptoms of extra-cellular-matrix (extracellular matrix) production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism is made to improve effectively, Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), The illness which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism can be treated effectively.
[0002]
[Description of the Prior Art]In recent years, the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem. With the illness which shows the symptoms of sthenia of extracellular matrix production here. For example, keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. is included.
[0003]For example, the liver cirrhosis said for the deceased to climb at least our country also in

about 20,000 people per year is the hepatic fibrosis of ******** covering the flume crack which is a sick general term which becomes hard since liver is growth of connective tissue, and is an end image of various chronic liver disease, and the whole liver. That is, in the chronic hepatitis which attains to a long period of time, liver injury, such as inflammation, carries out fibrosis of the liver with remarkable sthenia of extra-cellular-matrix (especially I-beam collagen) production of fibroblast, Ito cells, etc. If the fibrosis of a liver advances chronically, increasingly normal liver regeneration is blocked, hepatocytes are replaced, and the extra-cellular matrix which makes I-beam collagen fibroblast with a subject will occupy most portion of a hepatic tissue, and will result in the liver cirrhosis which consists of many ******. The abnormalities of the blood flow which a fiber septum progresses to the whole liver and produces as a result with advance of liver cirrhosis, It also becomes a cause which pushes the denaturation of a hepatocyte further, and the vicious circle in liver cirrhosis will continue, and also a lot of colloid fibers in liver are generated by various causes, such as alcohol, a virus, and autoimmunity, a necrosis and afunction of hepatocytes arise by them, and a cirrhotic patient dies finally. Although I-beam collagen occupies about 2% of a total protein amount in a normal liver, if it serves as liver cirrhosis, it will come to occupy 10 to 30%.

[0004]An interstitial lung disease is a disease group characterized by the chronic inflammation (alveolitis alveolitis) of an alveolus and not only an alveolar duct but the lower respiratory tract which also often involves in respiratory bronchiole and an end bronchial tube, the fibrosis of the stromata which are the result, and alveolar endogenous-fibers-ization. With an interstitial lung disease here, diffusion interstitial lung diseases, such as pneumonitis and fibroid lung, idiopathic pulmonary fibrosis, the penetrable pulmonary edema, a collagen disease of lung, sarcoidosis, etc. are included, for example. In an interstitial lung disease, superfluous production and accumulation of the extra-cellular matrix are accepted in the fibrosis organization. That is, in the lung fibrosis organization of an interstitial lung disease, accumulation of a Tsuguaki I-beam and III type collagen is seen by enlarged stromata, the alveolar septa which carried out thickening to the early stage of fibrosis are piled up, a stadium reaches an advanced stage, I-beam collagen increases in the second half, and especially III type collagen turns into main collagen. Basement membrane is destroyed at an early stage, and invasion of the collagen fiber by the side of an alveolar space is observed.

[0005]Chronic renal failure is in the state where it became impossible to maintain homeostasis in the living body by desolation of a kidney function, as a result of chronic nephritic syndrome. When advance of chronic renal failure is seen pathologically, it is advance of glomerulosclerosis and stromata fibrosis. The glomerulosclerosis is the hyperplasia of the extra-cellular matrix centering on a mesangium field. It compares that it is normal, and the ingredient of glomerular basement membrane, such as IV type collagen, increases to Tsuguaki, and the ingredient of mesangium sclerosis is carrying out hyperplasia also of the I-

beam collagen which is a stromata ingredient in accordance with the sclerosis part. That is, it is a factor with major production sthenia of an extra-cellular matrix to the glomerulosclerosis which passes in chronicity. With a ****** disease, IgA glomerulonephritis, focal glomerular sclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, a chronic interstitial nephritis, chronic glomerulonephritis, etc. are included in chronic renal failure here, for example.

[0006]A cardiac muscle cell is a cell which specialized highly, and does not have the capability with it to divide and increase. Therefore, if a certain load is added to the heart, the each will get fat, and a cardiac muscle cell will increase a shrinkage force, and will try to maintain cardiac performance. If load continues for a long time, a variegated obstacle will be accumulated focusing on the factor of the ischemia, cause a breakdown to the compensatory mechanism over load, the shrinkage force of a myocardium declines rapidly, and the pumping ability of the heart is spoiled -- cardiac insufficiency -- ****** -- things are known and cardiac hypertrophy occupies the biggest portion as the origin of the cardiac insufficiency in our country. The merger rate of ischemic heart disease or critical ventricular arrhythmia becomes high intentionally, and formation of cardiac hypertrophy not only becomes the greatest risk factor of the onset of cardiac insufficiency, but has become a factor which specifies a vital prognosis independently. In order that each cardiac muscle cell not only gets fat remarkably, but may bundle the cardiac muscle cell firmly at the time of cardiac hypertrophy progress, the fibrosis of stromata is promoted and collagen which is an extra-cellular matrix increases. If a cardiac muscle cell is lost by myocarditis, myocardial ischemia, etc., collagen will be biosynthesized and a gap will be replaced. If the fibrosis of stromata progresses superfluously as a result, a myocardium will become hard and the obstacle of the extension will be carried out. A muscle snug function also falls and the obstacle also of the relaxation of the diastole of a myocardium is carried out. In addition, the gastric upset of collagen synthesis happens by a certain cause, and the illnesses which show the symptoms of extracellular-matrix-production sthenia of a postoperative scar, a burn nature scar or a scleroderma, arteriosclerosis, etc. are considered to be the origins with main fibrosis progressing and producing hardening change of an organization.

[0007]Also in the vascularization, the collagen synthesis in basement membrane and basement membrane, An important role. Achieving is pointed out (). [Maragoudakis and] E., Sarmonika, M., and and. Panoutsacopoulous and M., "J. Pharmacol. Exp. Ther.", 244 : 729, 1988 ; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119: 1768, 1986. As a disease by the vascularization, for example Diabetic retinopathy, back fibrae-lentis vegetation, The vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, a trunk plug, the pyogenic granuloma, hemanglioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis,

various tumors, etc. are known. Thus, although the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem, In the former, extra-cellular-matrix synthetic inhibitor (for example, collagen synthesis depressant) should be satisfied with various fields, such as side effects and a medicinal value, of synthetic inhibitor was not yet developed.

[0008]On the other hand, a heat shock protein (it is also called heat shock protein;HSP and stress protein), a group revealed by the cell by stimulating a cell by a certain stress, for example, heat, a heavy metal, drugs, an amino acid analog, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally.

It is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

Although the kind is various, HSP, From the size of a molecular weight to HSP90 family (for example, HSP of 90kD or 110kD, etc.). It can divide roughly into 4 of HSP70 family, HSP(s)(for example, HSP of 70 - 73kD, etc.)60 family, and low molecule HSP(s) (for example, HSP of 57 - 68kD, etc.) families (for example, HSP of 20kD, 25 - 28kD, or 47kD, etc.) families. In this Description, the number indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 47kD shall be called "HSP47." As mentioned above, although many kinds exist in HSP, these differ not only in a molecular weight but in structure, a function, or character etc., respectively. the response to stress -- in addition, some of these protein being compounded compositionally and under normal environment, A thing like a meeting of proteinic folding, unfolding, and a protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown. These functions as a heat shock protein are called a molecular chaperone.

[0009]HSP47 is the protein discovered by Hitoshi Nagata in 1986, and is basic protein (pI=9.0) with a molecular weight of 47 kilodalton. As the manifestation of HSP47 increases, It is shown by various cells that composition of collagen also increases ("J. Biol. Chem."261: 7531 and 1986;" Eur. J. Biochem."206:323 and 1992;" J.). Biol. Chem.", 265 ; 992, 1990 ; "J. Clin. Invest.", 94:2481, 1994. Namely, HSP47 is an aspect of affairs of processing of the procollagen within an endoplasmic reticulum, 3 heavy-chain helix formation, or the procollagen transportation and secretion to Golgi apparatus from an endoplasmic reticulum in intracellular, Since it is functioning as a specific molecular chaperone of collagen, the HSP47 manifestation which increased stimulates accumulation of the collagen molecule in an extra-cellular matrix. Thus, HSP47 which is a collagen binding heat shock protein is the heat shock protein which related to collagen which is extracellular matrix protein closely also in the function like the manifestation.

[0010]

[Problem to be solved by the invention]This invention persons in view of the above-mentioned

situation Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), The keloid and the hypertrophic scar which are produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., The physiological status of the sick patient who shows the symptoms of extracellular-matrix-production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism is made to improve effectively, Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), The keloid and the hypertrophic scar which are produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., In order to provide the extra-cellular-matrix synthetic inhibitor which can treat effectively the illness which shows the symptoms of extracellular-matrix-production sthenia of a scleroderma, arteriosclerosis, or articular rheumatism, examination was come in piles variously.

[0011]As described above, liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), The symptoms which the extra-cellular matrix in an organ is remarkable, and fibrosing diseases produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., such as keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism, increased are understood to be the main lesion. Liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), It is thought that the fibrosis which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism and by which it is accompanied sick is produced due to the increase in collagen biosynthesis or the fall of collagen degradation ability. For example, in the fibrosis of a liver, although synthesis-activities-ization of an I-beam, an III type, and IV type collagen takes place, synthesis-activities-ization of I-beam collagen which is especially a basic component has an important meaning.

[0012]Under such a situation, this invention persons found out that the JINSENO sides which are ingredients, such as a ginseng or Ginseng Radix Rubra, also unexpectedly, especially JINSENO side Rg_1 controlled specifically composition of HSP47 in the cell of the organization which shows symptoms. Namely, composition of intracellular HSP47 is controlled by prescribing JINSENO sides for the patient, Control the collagen synthesis within an organ and As a result, liver cirrhosis, an interstitial lung disease, It found out that the sick therapy which shows the symptoms of extracellular-matrix-production sthenia of the keloid produced after chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism was possible. This invention is based on such knowledge and Liver cirrhosis, an interstitial lung disease, Chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, a postoperative scar and burn nature

scar, The keloid, the hypertrophic scar, the scleroderma, arteriosclerosis which are produced after a traffic accident etc., Or it is HSP47 synthetic inhibitor which can treat effectively the illness which shows the symptoms of sthenia of extracellular matrix production, such as articular rheumatism, the collagen which has played the role important for maturation and the transport process of intracellular collagen -- it aims at providing synthetic inhibitor of HSP47 which is a specific molecular chaperone.

[0013]

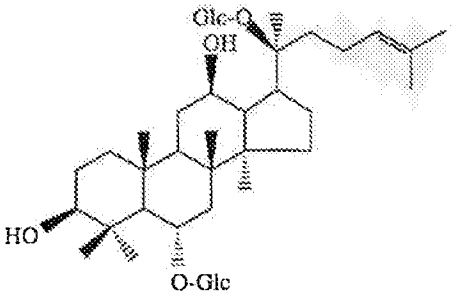
[Means for solving problem]Therefore, this invention relates to HSP47 synthetic inhibitor containing JINSENO sides, especially JINSENO side Rg_1 as an active principle.

[0014]

[Mode for carrying out the invention]Hereafter, this invention is explained in detail. Synthetic inhibitor of this invention contains JINSENO sides as an active principle. In this Description, with JINSENO sides (GINSENOSHIDO; ginsenosides). For example, the JINSENO side Ro (GINSENOSHIDO Ro;ginsenoside Ro; chikusetsu-saponin V;chikusetsusaponinV; saponin A;saponin A), JINSENO side Ra_1 (GINSENOSHIDORa_1;ginsenoside Ra_1), JINSENO side Ra_2 (GINSENOSHIDORa_2;ginsenoside Ra_2), JINSENO side Rb_1 (GINSENOSHIDORb_1;ginsenoside Rb_1; saponin D;saponin D), JINSENO side Rb_2 (GINSENOSHIDORb_2;ginsenoside Rb_2), JINSENO side Rb_3 (GINSENOSHIDORb_3;ginsenoside Rb_3), The JINSENO side Rc (GINSENOSHIDO Rc;ginsenoside Rc), The JINSENO side Rd (GINSENOSHIDO Rd;ginsenoside Rd), JINSENO side Re (GINSENOSHIDO Re;ginsenoside Re), JINSENO side Rf (GINSENOSHIDO Rf;ginsenosideRf). JINSENO side Rg_1 (GINSENOSHIDORg_1;ginsenoside Rg_1), JINSENO side Rg_2 (GINSENOSHIDORg_2;ginsenoside Rg_2; chikusetsu-saponin I;chikusetsusaponinI), JINSENO side Rg_3 (GINSENOSHIDORg_3;ginsenosideRg$_3$), JINSENO side Rh_1 (GINSENOSHIDORh_1;ginsenoside Rh_1), JINSENO side Rh_2 (GINSENOSHIDORh_2;ginsenoside Rh_2), 20-guru KOJINSENO side Rf (20-guru KOGINSENOSHIDO Rf;20-glucoginsenoside Rf), Malonyl JINSENO side Rb_1 (malonyl GINSENOSHIDORb_1;maronylginsenoside Rb_1), Malonyl JINSENO side Rb_2 (malonyl GINSENOSHIDORb_2;maronylginsenoside Rb_2), the malonyl JINSENO side Rc (malonyl GINSENOSHIDORc;maronylginsenosideRc), The malonyl JINSENO side Rd (malonyl GINSENOSHIDO Rd;maronylginsenoside Rd), The chikusetsu-saponin Ia (chikusetsusaponin Ia), The chikusetsu-saponin Ib (chikusetsusaponin Ib), The chikusetsu-saponin III (chikusetsusaponin III), The chikusetsu-saponin IV (chikusetsusaponinIV; saponin B;saponin

B), The chikusetsu-saponin IVa (chikusetsusaponinIVa; saponin C;saponin C), Protopanaxadiol (protopanaxadiol), pro TOPANAKISA triol (protopanaxatriol), oleanolic acid (oleanolic acid), etc. mean the stereoisomeric form of these compounds. In this invention, those JINSENO sides can also be simultaneously used combining several JINSENO sides which can also use independently or are different.

[0015]Especially as JINSENO sides which can be used as an active principle in synthetic inhibitor of this invention, JINSENO side Rg_1 is the most preferred. JINSENO side Rg_1 (GINSENOSHIDORg_1;ginsenoside Rg_1) is formula (I). : [Chemical formula 1]



(I)

It is expressed with (the inside of a formula and Glc are beta-D-glucopyranosyl groups), and it is a compound of molecular formula $C_{42}H_{72}O_{14}$ and the molecular weight 801.03, for example, is contained in crude drugs, such as a ginseng or Ginseng Radix Rubra.

[0016]The JINSENO sides contained in synthetic inhibitor of this invention can be prepared chemosynthesis or by extracting from a natural product and refining. Or a commercial item may be used. In extracting the JINSENO sides used as an active principle in synthetic inhibitor of this invention from a natural product, For example, it extracts using what (for example, crude drug) carried out processing treatment (for example, desiccation, cutting, parboiling, steam heating, or disintegration) simply using vegetable [containing JINSENO sides / the whole or some (for example, the entire plant, a leaf, a root a rhizome a stem a cortex or a flower) of] as it is. If extraction conditions are conditions generally used for vegetable extraction, there will be no restriction in particular. As vegetation containing JINSENO sides, although it does not limit to this, they are araliaceous (Araliaceae) Panax schinseng (Panax ginseng C.A.Meyer;Panax schinseng Nees) and Panax japonicus, for example. [Panaxjaponicus C.A.Meyer;Panax schinsengNees var.japonicum Makino;Panax pseudo-ginseng (Will.) subsp. japonicus Hara] SANSHI thynnine gin [Panax notoginseng (Burkill) F.H.Chen;Panax sanchi Hoo;Panaxpseudo-ginseng Wallich var.notoginseng (Burkill) Hoo et Tseng] Or a U.S. ginseng (Panax quinquefolium L.) etc. can be used.

[0017]When extracting the JINSENO sides in this invention from a crude drug, it does not limit to this, but it is preferred to extract from a ginseng or Ginseng Radix Rubra for example. a ginseng (ginseng radix: Ginseng Radix) means what passed lightly the root or this except the

rootlet of Panax schinseng through hot water, and is independent about those portions -- it is -- it can be used, combining arbitrarily. Ginseng Radix Rubra (red ginseng; Red Ginseng;Ginseng Radix Rubra) means what steamed the root of Panax schinseng, and is independent about those portions -- it is -- it can be used, combining arbitrarily.

[0018]The ginseng extract or the Ginseng Radix Rubra extract which can be used as an active principle in synthetic inhibitor by this invention should just contain the aforementioned JINSENO sides, especially JINSENO side Rg_1, therefore the crude extract of a ginseng or Ginseng Radix Rubra can be used for it. It can obtain by water's (for example, chilled water's, warm water's, preferably boiling water's) extracting a ginseng or Ginseng Radix Rubra, or extracting it using an organic solvent as a manufacturing method of the ginseng extract which can be used by this invention, or the Ginseng Radix Rubra extract. as an organic solvent -- alcohol (for example, methyl alcohol.) of the carbon numbers 1-6 Ethyl alcohol, n-propyl alcohol, isopropyl alcohol, or butyl alcohol and ester (for example, methyl acetate and ethyl acetate.) Propyl acetate or butyl acetate, ketone (for example, acetone or methyl isobutyl ketone), Ether, petroleum ether, n-hexane, cyclohexane, toluene, the halogen derivative (for example, a carbon tetrachloride and dichloromethane.) of benzene and hydrocarbon or chloroform, pyridine, and a glycol (for example, propylene glycol.) Or a butylene glycol, a polyethylene glycol, or acetonitrile can be used, and these organic solvents can be mixed by independence or proper combination, and a fixed ratio, and also it can use by anhydrous or a moisture state. Preferably, methyl alcohol etc. are desirable. Being able to use the method used for the usual crude drug extraction as the method of water extraction or organic solvent extraction, for example, stirring to ginseng (desiccation) or Ginseng Radix Rubra 1 weight section using three to water or organic solvent 300 weight section. It is desirable at the temperature below the boiling point ultrasonic extraction or to carry out maceration at heating flowing back and ordinary temperature. The extraction process can shorten extraction time by carrying out preferably for 10 minutes - 24 hours, and usually adding supplementary means, such as stirring, for 5 minutes - seven days, if needed.

[0019]After the end of an extraction process, by suitable methods, such as filtration or centrifugal separation, an insoluble matter can be separated and a crude extract can be obtained from water or an organic solvent extract. In synthetic inhibitor of this invention, it may be used as it is, without refining especially the aforementioned crude extract, in using extraction, the JINSENO sides which carried out fractionation, especially JINSENO side Rg_1 from a natural product. The refining extracts processed further can also use the aforementioned crude extract other than the water extract by a conventional method, or an organic solvent extract as an active principle of synthetic inhibitor of this invention with various organic solvents or adsorbent. The ginseng extract or the Ginseng Radix Rubra extract containing the refining extracts which finished such crude extract and various kinds of

purification treatment, Even if it uses the extracted solution, the extract which condensed the solvent may be used, and the powder which distilled off the solvent and was dried and also the thing crystallized and refined, or a viscous substance may be used, and those diluents can also be used. In this way, the obtained ginseng extract or the Ginseng Radix Rubra extract contains the impurity which originates in the ginseng or Ginseng Radix Rubra of a raw material simultaneously, including as a mixture the JINSENO sides contained in a ginseng or Ginseng Radix Rubra.

[0020]The vegetable extract in which synthetic inhibitor of this invention contains JINSENO sides or JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially) about a ginseng extract or the Ginseng Radix Rubra extract, it is independent [its] -- or -- desirable -- galenical pharmacy -- the usual carrier permissible-like or in veterinary medicine -- an animal -- mammalian (especially Homo sapiens) can be medicated preferably. Especially as an administration pharmaceutical form, there is no limitation and For example, powder medicine, subtle granules, a granule, Parenterals, such as oral agents, such as a tablet, a capsule, suspension, an emulsion agent, syrups, extracts, or a pill, or injections, liquids for external use, an ointment, suppositories, cream of local administration, or eye drops, can be mentioned. These oral agents, for example Gelatin, sodium alginate, starch, Cornstarch, white soft sugar, milk sugar, grape sugar, mannite, carboxymethyl cellulose, Dextrin, a polyvinyl pyrrolidone, crystalline cellulose, a soybean lecithin, Sucrose, fatty acid ester, talc, magnesium stearate, a polyethylene glycol, In accordance with a conventional method, it can manufacture using excipients, such as a magnesium silicate, a silicic acid anhydride, or synthetic aluminum silicate, a binding material, disintegrator, a surface-active agent, lubricant, a fluid accelerator, a diluent, a preservative, colorant, perfume, corrigent, a stabilizing agent, a moisturizer, an antiseptic, or an antioxidant. For example, it is the capsule etc. which were mixed and filled up with JINSENO side Rg_1 of one weight section, and milk sugar of 99 weight sections.

[0021]As the parenteral administration method, injection (inside of hypodermic and a vein, etc.) or rectum administration is illustrated. In these, injections are used most suitably. In preparation of injections, for example, the JINSENO sides (especially JINSENO side Rg_1) as an active principle. (Or the extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) Besides a ginseng extract or the Ginseng Radix Rubra extract, for example Water soluble solvents, such as a physiological saline or Ringer's solution, Isotonizing agents, such as nonaqueous solubility solvents, such as vegetable oil or fatty acid ester, grape sugar, or sodium chloride, a solubilizing agent, a stabilizing agent, an antiseptic, a suspending agent, or an emulsifier can be used arbitrarily. Synthetic inhibitor of this invention may be prescribed for the patient using the technique of a sustained release drug in which the controlled-release polymer etc. were

used. For example, synthetic inhibitor of this invention can be made to be able to incorporate into the pellet of ethylene vinyl polymer acetate, and it can transplant surgically during the organization which should treat this pellet.

[0022]Although synthetic inhibitor of this invention is not limited to this, it can contain JINSENO sides in 0.1 to 80weight % of quantity preferably 0.01 to 99weight %. (The extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) Synthetic inhibitor of this invention which contains a ginseng extract or the Ginseng Radix Rubra extract as an active principle can be suitably adjusted so that the JINSENO sides (especially JINSENO side Rg_1) contained in it may become the aforementioned quantity range, and it can be prepared. (The extract of the vegetation containing JINSENO sides, for example, the extract of the crude drug containing JINSENO sides, (especially)) It is preferred to pharmaceutical-preparation-ize it using a carrier permissible in galenical pharmacy, in considering the synthetic inhibitor which contains a ginseng extract or the Ginseng Radix Rubra extract as an active principle as the pharmaceutical preparation for internal use.

[0023]The dose in the case of using synthetic inhibitor of this invention, Although it changes with a sick kind, a patient's age, sex, weight, the grade of condition, or medication methods and there is no restriction in particular, about 1mg-10g is usually prescribed [as an amount of JINSENO side Rg_1] for the patient in taking orally or parenterally in about 1 to 4 steps per day per adult. It is also possible for a use not to be limited to drugs, either and to give it in the form of ingesta as various uses, for example, functional food, and health food.

[0024]

[Function]As described above, the JINSENO sides contained in synthetic inhibitor of this invention, especially JINSENO side Rg_1, Since there is an operation which controls HSP47 intracellular composition specifically, if said JINSENO sides are prescribed for the patient, HSP47 intracellular biosynthesis will decrease specifically and the biosynthesis of collagen will be controlled. As a result, extracellular matrix production will also be controlled. Therefore, the illness which shows the symptoms of the extracellular-matrix-production sthenia accompanied by the increase in collagen in said JINSENO sides, For example, it can be used for the prevention and the therapy of keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism which are produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. That is, synthetic inhibitor of this invention controls composition of collagen by controlling composition of HSP47 which is a collagen specific chaperon.

[0025]Since playing a role with important collagen synthesis in basement membrane and

basement membrane is pointed out, also in the vascularization, as mentioned above synthetic inhibitor of this invention, Are very useful as a prevention remedy of many diseases based on the plague of the vascularization, Each disease which was described previously, i.e., diabetic retinopathy, back fibrae-lentis vegetation, It can use for the vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, the pyogenic granuloma that cannot be dried, hemanglioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis, various tumors, etc. Since it is clear to achieve a guiding role until the cancer cell in which the stromata (interstitial stroma) made into a basic skeleton furthermore seceded from I-beam collagen and fibronectin in metastasis of cancer invades into a nearby vessel ["BIOTHERAPY", 7 (8) : 1181, 1993] By prescribing synthetic inhibitor of this invention for the patient, it is also possible to control metastasis of cancer.

[0026]

[Working example]Hereafter, although an embodiment explains this invention concretely, these do not limit the range of this invention.

Embodiment 1: Peptide which consists of 15 amino acid corresponding to the amino terminal of preparation Homo sapiens HSP47 of the production (1) anti-HSP47 polyclonal antibody of anti-HSP47 polyclonal antibody to the 2-16th amino acid sequences It produces using the automatic peptide synthesis equipment (PSSM-8 system, the Shimazu work place) [hereafter called Homo sapiens HSP47 peptide (2-16)], and is SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate. Using [SMPB:Succinimidyl 4-(p-maleimidophenyl) butyrate] as a cross linking agent, it was made to combine with lactoglobulin with a conventional method ("Biochemistry", 18:690, 1979), and the sensitized antigen was produced. Phosphate buffered saline containing this sensitized-antigen 150mug [presentation: :Cosmobio which calls below KCl=0.2 g/l, KH_2PO_4=0.2 g/l, NaCl=8 g/l, and Na_2HPO_4(anhydrous) =1.15-g/l:PBS (-), catalog number 320-01] The hypodermic of the roux rat (6-week old, feminity: CLEA Japan) was medicated with 0.2 ml of mixed liquor obtained by mixing 0.2 ml and equivalent weight of Freund's complete adjuvant (YATORON, catalog number RM606-1), and immunity was carried out. After repeating second and the 3rd immunity in a similar way, immunization was performed 6 times using the adjuvant (Hunter's TiterMax; CytRx Corporation, Georgia, U.S.). It collected blood from the sensitization animal, and the blood serum was separated with the conventional method, it extracted, and the antibody titer in a blood serum was measured by the enzyme-labeled antibody technique (the ELISA method) and Western blot technique which are shown below.

[0027](2) Homo sapiens HSP47 peptide (2-16) prepared for the evaluation preceding clause (1) of the anti-HSP47 polyclonal-antibody characteristic by an enzyme-labeled antibody technique (the ELISA method) is dissolved in PBS (-), The peptide solution of 10 microg/ml

concentration was prepared, and said peptide solution was dropped at each well of the rigid ASEI plate (a falcon, the catalog number 3910) every [50micro / l]. After putting only 50micro of PBS(-) I into the outermost well and neglecting it at 4 ** under humid overnight, After throwing away said peptide solution and washing each well using PBS (-), 100micro of PBS(-) I which contains bovine serum albumin (it is hereafter called BSA for short) 1% was put into each well, and it was neglected under the room temperature for 1 hour. After washing 3 times by PBS (-), roux rat blood serum 50mul acquired for the preceding clause (1) was put into each well, and it allowed to stand at the room temperature for 1 hour. After washing 3 times by PBS (-), peroxidase-labeling anti-rat IgG50microl was put into each well as a secondary antibody, and it was neglected under the room temperature for 1 hour. After washing twice by PBS (-), The substrate liquid every I [100mu] which dissolved and prepared one o-phenylenediamine (OPD) tablet (a sigma, the catalog number P8287) (10 mg) to 10 ml of 0.1M citrate buffers (pH 4.5) which added hydrogen-peroxide-solution 4mul is dropped at each well, After allowing to stand for 30 minutes under protection from light at a room temperature, the absorbance of 492 nm of each well was measured with the microplate leader (TOSOH, MPR-A4i type). The blood serum with which the rise of antibody titer was checked was used as the anti human HSP47 polyclonal antibody, and was used for the following embodiments.

[0028](3) The buffer system (Laemmli, N. K., "Nature", 283:pp. 249-256, 1970) of the evaluation Laemmli of the anti-HSP47 polyclonal-antibody characteristic by a Western blot technique is used, Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis of the lysate of a HeLa cell was performed in accordance with the following methods. Preparation of concentrating gel was performed as follows. 6.1 ml of distilled water, 0.5M tris (Bio-Rad, catalog number 161-0716) - HCl(pH 6.8) 2.5ml, 10%SDS(Bio-Rad, catalog number 161-0301) 100microl, And 1.3 ml of 30% acrylamide (Bio-Rad, catalog number 161-0101) / N,N'-methylenebis acrylamide (Bio-Rad, catalog number 161-0201) are mixed, Carry out indirect desulfurization mind for 15 minutes, and 10% ammonium persulfate (Bio-Rad, catalog number 161-0700) 50microl and N,N,N',N'-tetramethylethylenediamine. (It is hereafter called TEMED for short) 10microl -- in addition, concentrating gel was prepared (Bio-Rad, catalog number 161-0800). Preparation of resolving gel was performed as follows. 4.045 ml of distilled water, 1.5M tris-HCl(pH 8.8) 2.5ml, 10%SDS100microl, and 3.3 ml of 30% acrylamide / N,N'-methylenebis acrylamide are mixed slowly, It degassed with the aspirator for 15 minutes, and 10% ammonium persulfate 50mul and TEMED5microl were added. As a migration buffer, distilled water was added to 9.0 g of tris, 43.2 g of glycines (Bio-Rad, catalog number 161-0717), and SDS3.0g, it was made 600 ml, and what diluted this with distilled water 5 times was used. A sample buffer 2 ml of distilled water, 2M tris-HCl(pH 6.8) 500microl, What mixed SDS0.32g and beta-mercaptoethanol 800microl and 0.05% (w/v) bromophenol-blue (Bio-Rad, catalog number 161-0404) 400microl was used.

[0029]Under 5% carbon dioxide conditions, at 37 **, the HeLa cell was cultured in the MEM culture medium which contains inactivation fetal calf serum (it is hereafter called FBS for short) 10%, and the lysate was prepared. After performing SDS-polyacrylamide gel electrophoresis of the obtained HeLa-cell lysate, Gel is stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), The room temperature performed blotting by 100V for 3 hours using protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad). As a blotting buffer. ** 0.025M tris. And the trisglycine buffer which consisted of 0.192M glycine and was adjusted the pH to 8.5 () [Tris Gly Running and Blotting Buffer;Enprotech and] The U.S. Massachusetts state and the buffer which prepared methyl alcohol in addition to catalog number SA100034 so that it might become 20% were used. It blocked by dipping a nitrocellulose membrane in the PBS (-) solution which contains skim milk (Snow Brand Milk Products) 5% for 30 minutes at a room temperature after blotting. Primary antibody reactions were performed after blocking using the screener blotter (sampler tech) by making into a primary antibody the roux rat blood serum acquired for the preceding clause (1). It is said roux rat blood serum 200mul diluted with PBS (-) which contains skim milk (Snow Brand Milk Products) 2% 10 times, and primary antibody reactions were performed for 120 minutes at the room temperature. The shake for 5 minutes by PBS (-) after primary antibody ending reaction using a slow locking shaker 2 times, The nitrocellulose membrane was washed by the PBS (-) solution which contains Tween20 (Bio-Rad, catalog number 170-6531) 0.1% performing shake for 15 minutes, and performing shake for 5 minutes twice by 4 times and also PBS (-). Secondary antibody reactions were performed after the end of washing for 2 hours using 5 ml of solutions which diluted the peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) with the PBS (-) solution which contains skim milk 2% 5000 times. The PBS (-) solution and the PBS (-) solution which contains Tween20 0.1% washed the nitrocellulose membrane under the same conditions as washing after primary antibody reactions after ending reaction.

[0030]After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Western blotting detection reagent; Amersham, catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After settling at a room temperature for 1 minute, an excessive detecting reagent is removed, a nitrocellulose membrane is wrapped in a lap, and a reaction surface was stuck to an X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), and was made to expose. Examination of the reactivity of anti-HSP47 polyclonal antibody was performed after development by measuring a band near [equivalent to HSP47] a molecular weight of 47 kilodalton. A blood serum with which a rise of antibody titer was checked was used for the following embodiments as anti human HSP47 polyclonal antibody.

[0031]Embodiment 2 : culture human breast cancer cell stock MCF7 (ATCC HTB 22) of a measurement (1) Homo sapiens cultured cancer cell of a HSP expression amount of the Homo

sapiens cultured cancer cell, In RPMI1640 culture medium including 10^{-8}Mbeta-estradiol and the 10% inactivation FBS, it cultivated at 37 ** under 5% carbon dioxide conditions except the time of heat shock processing.

[0032](2) JINSENO side Rg_1 (Matsuura Yakugyo) expressed with said formula (I) that it becomes the last concentration M of 100micro in a culture medium of said human breast cancer cell stock MCF7 two days after JINSENO side Rg_1 processing and heat shock processing seeding was added, and it cultivated for 24 hours. Then, after carrying out heat shock processing for 15 minutes at 45 **, it cultivated at 37 ** all night. A control test was carried out like the above except not adding JINSENO side Rg_1.

[0033](3) Each cell processed for the measurement preceding clause (2) of the HSP expression amount in the Homo sapiens cultured cancer cell was homogenized by the method shown below, and the HSP expression amount was measured in the Western blot technique. Namely, the lysis buffer (lysis buffer) after washing the cell processed for the preceding clause (2) by PBS (-) [1.0%NP-40, 0.15M sodium chloride, the 50mM tris- HCl (pH 8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonylfluoride, 2 microg [/ml] leupeptin, and 2 microg [/ml] pepstatin] 1 ml was added and it settled for 20 minutes in Hikami. Then, centrifugality was performed for 20 minutes at 12000 rpm at 4 **. 10micro of supernatant liquid I after centrifugality was added to 790micro of PBS(-) I, and also 200micro of protein assay stain solutions (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) I were added. For 5 minutes, after settling at a room temperature, the absorbance was measured at 595 nm and protein quantification was performed. The SDS polyacrylamide gel electrophoresis of the lysate which contains equivalent weight of protein by the buffer system of Laemmli was performed using the sample which performed protein quantification. In accordance with the method described in Embodiment 1, blocking following blotting and it was performed after the electrophoresis. Namely, protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad) is used, At the room temperature, gel was stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. As a blotting buffer, the same thing as the buffer used in said Embodiment 1 (3) was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific combination was blocked.

[0034]An anti human HSP47 rat polyclonal antibody manufactured in Embodiment 1 performed primary antibody reactions on a nitrocellulose membrane after blocking. Then, exchange every [a for / 5 minutes], and a solution by PBS (-), and a slow locking shaker performs two washing, Every [a for / 15 minutes] and a solution were exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS(-)-0.1%, and four washing was performed. Eventually,

every [a for / 5 minutes] two washing was performed by PBS (-). A peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) after an end of washing, Secondary antibody reactions were performed for 2 hours using 5 ml of antibody solutions diluted and prepared 5000 times with an PBS (-) solution which contains skim milk 2%. After ending reaction, about a nitrocellulose membrane, a solution was changed for 5 minutes at a time with an PBS (-) solution, a solution was changed for 15 minutes at a time with Tween20 solution 2 times and also PBS(-)-0.1%, and a slow locking shaker performed five washing. Finally an PBS (-) solution performed every [a for / 5 minutes] two washing. After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Westernblotting detection reagent;Amersham and catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After incubating for 1 minute, an excessive detecting reagent was removed, a nitrocellulose membrane was wrapped in a lap, a reaction surface was stuck to an X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), was exposed, was developed, and existence of HSP47 was examined.

[0035]In breast cancer cell line MCF7 which did not add a control test, i.e., JINSENO side Rg_1, the band of molecular weight abbreviation 47kD was detected one. In breast cancer cell line MCF7 which added JINSENO side Rg_1, the corresponding band was a very thin band. That is, JINSENO side Rg_1 can be concluded to have the activity of the synthetic inhibitor which controls the manifestation of HSP47, and this fact shows that JINSENO side Rg_1 works restrainedly to sthenia of extracellular matrix production.

[0036]

[Effect of the Invention]As explained in full detail above, synthetic inhibitor of this invention, For example, liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), It has the operation which improves the collagen synthesis sthenia seen by the cell which shows the symptoms of sthenia of extracellular matrix production, such as keloid produced after cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc., a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism, and which fell ill sick. Therefore, by prescribing synthetic inhibitor by this invention for the patient, an organ, the fibrosis of an organization, and hardening can be prevented, as a result, said sick patient's physiological status can be made to be able to improve effectively, and said illness can be treated effectively. Synthetic inhibitor of this invention is useful also for the prevention therapy of the various diseases accompanied by the plague of the vascularization. Since it is clear to achieve a guiding role until the cancer cell in which the stromata made into a basic skeleton seceded from I-beam collagen and fibronectin in metastasis of cancer invades into a nearby vessel, it is also possible by prescribing synthetic inhibitor of this invention for the patient to control metastasis of cancer.

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* NOTICES *

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PRIOR ART

[Description of the Prior Art]In recent years, the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem. With the illness which shows the symptoms of sthenia of extracellular matrix production here. For example, keloid, a hypertrophic scar, a scleroderma, arteriosclerosis, or articular rheumatism produced after liver cirrhosis, an interstitial lung disease, chronic renal failure (or chronic renal failure ****** disease), cardiac hypertrophy, a postoperative scar and a burn nature scar, a traffic accident, etc. is included.
[0003]For example, the liver cirrhosis said for the deceased to climb at least our country also in about 20,000 people per year is the hepatic fibrosis of ******** covering the flume crack which is a sick general term which becomes hard since liver is growth of connective tissue, and is an end image of various chronic liver disease, and the whole liver. That is, in the chronic hepatitis which attains to a long period of time, liver injury, such as inflammation, carries out fibrosis of the liver with remarkable sthenia of extra-cellular-matrix (especially I-beam collagen) production of fibroblast, Ito cells, etc. If the fibrosis of a liver advances chronically, increasingly normal liver regeneration is blocked, hepatocytes are replaced, and the extra-cellular matrix which makes I-beam collagen fibroblast with a subject will occupy most portion of a hepatic tissue, and will result in the liver cirrhosis which consists of many ******. The abnormalities of the blood flow which a fiber septum progresses to the whole liver and produces as a result with advance of liver cirrhosis, It also becomes a cause which pushes the denaturation of a hepatocyte further, and the vicious circle in liver cirrhosis will continue, and also a lot of colloid fibers in liver are generated by various causes, such as alcohol, a virus, and autoimmunity, a necrosis and afunction of hepatocytes arise by them, and a cirrhotic patient dies finally. Although I-beam collagen occupies about 2% of a total protein amount in a normal liver, if it serves as liver cirrhosis, it will come to occupy 10 to 30%.
[0004]An interstitial lung disease is a disease group characterized by the chronic inflammation

(alveolitis alveolitis) of an alveolus and not only an alveolar duct but the lower respiratory tract which also often involves in respiratory bronchiole and an end bronchial tube, the fibrosis of the stromata which are the result, and alveolar endogenous-fibers-ization. With an interstitial lung disease here, diffusion interstitial lung diseases, such as pneumonitis and fibroid lung, idiopathic pulmonary fibrosis, the penetrable pulmonary edema, a collagen disease of lung, sarcoidosis, etc. are included, for example. In an interstitial lung disease, superfluous production and accumulation of the extra-cellular matrix are accepted in the fibrosis organization. That is, in the lung fibrosis organization of an interstitial lung disease, accumulation of a Tsuguaki I-beam and III type collagen is seen by enlarged stromata, the alveolar septa which carried out thickening to the early stage of fibrosis are piled up, a stadium reaches an advanced stage, I-beam collagen increases in the second half, and especially III type collagen turns into main collagen. Basement membrane is destroyed at an early stage, and invasion of the collagen fiber by the side of an alveolar space is observed.

[0005]Chronic renal failure is in the state where it became impossible to maintain homeostasis in the living body by desolation of a kidney function, as a result of chronic nephritic syndrome. When advance of chronic renal failure is seen pathologically, it is advance of glomerulosclerosis and stromata fibrosis. The glomerulosclerosis is the hyperplasia of the extra-cellular matrix centering on a mesangium field. It compares that it is normal, and the ingredient of glomerular basement membrane, such as IV type collagen, increases to Tsuguaki, and the ingredient of mesangium sclerosis is carrying out hyperplasia also of the I-beam collagen which is a stromata ingredient in accordance with the sclerosis part. That is, it is a factor with major production sthenia of an extra-cellular matrix to the glomerulosclerosis which passes in chronicity. With a ****** disease, IgA glomerulonephritis, focal glomerular sclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, a chronic interstitial nephritis, chronic glomerulonephritis, etc. are included in chronic renal failure here, for example.

[0006]A cardiac muscle cell is a cell which specialized highly, and does not have the capability with it to divide and increase. Therefore, if a certain load is added to the heart, the each will get fat, and a cardiac muscle cell will increase a shrinkage force, and will try to maintain cardiac performance. If load continues for a long time, a variegated obstacle will be accumulated focusing on the factor of the ischemia, cause a breakdown to the compensatory mechanism over load, the shrinkage force of a myocardium declines rapidly, and the pumping ability of the heart is spoiled -- cardiac insufficiency -- ****** -- things are known and cardiac hypertrophy occupies the biggest portion as the origin of the cardiac insufficiency in our country. The merger rate of ischemic heart disease or critical ventricular arrhythmia becomes high intentionally, and formation of cardiac hypertrophy not only becomes the greatest risk factor of the onset of cardiac insufficiency, but has become a factor which specifies a vital prognosis

independently. In order that each cardiac muscle cell not only gets fat remarkably, but may bundle the cardiac muscle cell firmly at the time of cardiac hypertrophy progress, the fibrosis of stromata is promoted and collagen which is an extra-cellular matrix increases. If a cardiac muscle cell is lost by myocarditis, myocardial ischemia, etc., collagen will be biosynthesized and a gap will be replaced. If the fibrosis of stromata progresses superfluously as a result, a myocardium will become hard and the obstacle of the extension will be carried out. A muscle snug function also falls and the obstacle also of the relaxation of the diastole of a myocardium is carried out. In addition, the gastric upset of collagen synthesis happens by a certain cause, and the illnesses which show the symptoms of extracellular-matrix-production sthenia of a postoperative scar, a burn nature scar or a scleroderma, arteriosclerosis, etc. are considered to be the origins with main fibrosis progressing and producing hardening change of an organization.

[0007]Also in the vascularization, the collagen synthesis in basement membrane and basement membrane, An important role. Achieving is pointed out (). [Maragoudakis and] E., Sarmonika, M., and and. Panoutsacopoulous and M., "J. Pharmacol. Exp. Ther.", 244 : 729, 1988 ; Ingber, D. E., Madri, J. A., and Folkman, J., "Endocrinology", 119: 1768, 1986. As a disease by the vascularization, for example Diabetic retinopathy, back fibrae-lentis vegetation, The vascularization accompanying a corneal transplantation, glaucoma, an eye tumor, trachoma, a trunk plug, the pyogenic granuloma, hemanglioma, angioma fibrosum, a tumescent scar, granulation, rheumatism nature arthritis, the scleredema, atherosclerosis, various tumors, etc. are known. Thus, although the illness which shows the symptoms of sthenia of production of extra-cellular matrices, such as collagen, poses a big problem, In the former, extra-cellular-matrix synthetic inhibitor (for example, collagen synthesis depressant) should be satisfied with various fields, such as side effects and a medicinal value, of synthetic inhibitor was not yet developed.

[0008]On the other hand, a heat shock protein (it is also called heat shock protein;HSP and stress protein), a group revealed by the cell by stimulating a cell by a certain stress, for example, heat, a heavy metal, drugs, an amino acid analog, or hypoxia (low concentration oxygen) -- it is protein. The heat shock protein exists in the nature universally.

It is produced by bacteria, yeast, vegetation, an insect, and the higher animal including Homo sapiens.

Although the kind is various, HSP, From the size of a molecular weight to HSP90 family (for example, HSP of 90kD or 110kD, etc.). It can divide roughly into 4 of HSP70 family, HSP(s)(for example, HSP of 70 - 73kD, etc.)60 family, and low molecule HSP(s) (for example, HSP of 57 - 68kD, etc.) families (for example, HSP of 20kD, 25 - 28kD, or 47kD, etc.) families. In this Description, the number indicated to be HSP immediately after that shall show HSP which has specific molecular weight, for example, HSP of molecular weight 47kD shall be called

"HSP47." As mentioned above, although many kinds exist in HSP, these differ not only in a molecular weight but in structure, a function, or character etc., respectively. the response to stress -- in addition, some of these protein being compounded compositionally and under normal environment, A thing like a meeting of proteinic folding, unfolding, and a protein subunit and proteinic membrane transport for which the indispensable physiological role is played is shown. These functions as a heat shock protein are called a molecular chaperone. [0009]HSP47 is the protein discovered by Hitoshi Nagata in 1986, and is basic protein (pI=9.0) with a molecular weight of 47 kilodalton. As the manifestation of HSP47 increases, It is shown by various cells that composition of collagen also increases ("J. Biol. Chem."261: 7531 and 1986;" Eur. J. Biochem."206:323 and 1992;" J.). Biol. Chem.", 265 : 992, 1990 ; "J. Clin. Invest.", 94:2481, 1994. Namely, HSP47 is an aspect of affairs of processing of the procollagen within an endoplasmic reticulum, 3 heavy-chain helix formation, or the procollagen transportation and secretion to Golgi apparatus from an endoplasmic reticulum in intracellular, Since it is functioning as a specific molecular chaperone of collagen, the HSP47 manifestation which increased stimulates accumulation of the collagen molecule in an extra-cellular matrix. Thus, HSP47 which is a collagen binding heat shock protein is the heat shock protein which related to collagen which is extracellular matrix protein closely also in the function like the manifestation.

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EXAMPLE

[Working example]Hereafter, although an embodiment explains this invention concretely, these do not limit the range of this invention.
Embodiment 1: Peptide which consists of 15 amino acid corresponding to the amino terminal of preparation Homo sapiens HSP47 of the production (1) anti-HSP47 polyclonal antibody of anti-HSP47 polyclonal antibody to the 2-16th amino acid sequences It produces using the automatic peptide synthesis equipment (PSSM-8 system, the Shimazu work place) [hereafter called Homo sapiens HSP47 peptide (2-16)], and is SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate. Using [SMPB:Succinimidyl 4-(p-maleimidophenyl) butyrate] as a cross linking agent, it was made to combine with lactoglobulin with a conventional method ("Biochemistry", 18:690, 1979), and the sensitized antigen was produced. Phosphate buffered saline containing this sensitized-antigen 150mug [presentation: :Cosmobio which calls below KCl=0.2 g/l, KH_2PO_4=0.2 g/l, NaCl=8 g/l, and Na_2HPO_4(anhydrous) =1.15-g/l:PBS (-), catalog number 320-01] The hypodermic of the roux rat (6-week old, feminity: CLEA Japan) was medicated with 0.2 ml of mixed liquor obtained by mixing 0.2 ml and equivalent weight of Freund's complete adjuvant (YATORON, catalog number RM606-1), and immunity was carried out. After repeating second and the 3rd immunity in a similar way, immunization was performed 6 times using the adjuvant (Hunter's TiterMax; CytRx Corporation, Georgia, U.S.). It collected blood from the sensitization animal, and the blood serum was separated with the conventional method, it extracted, and the antibody titer in a blood serum was measured by the enzyme-labeled antibody technique (the ELISA method) and Western blot technique which are shown below.
[0027](2) Homo sapiens HSP47 peptide (2-16) prepared for the evaluation preceding clause (1) of the anti-HSP47 polyclonal-antibody characteristic by an enzyme-labeled antibody technique (the ELISA method) is dissolved in PBS (-), The peptide solution of 10 microg/ml concentration was prepared, and said peptide solution was dropped at each well of the rigid

ASEI plate (a falcon, the catalog number 3910) every [50micro / l]. After putting only 50micro of PBS(-) l into the outermost well and neglecting it at 4 ** by humid Shimo overnight, After throwing away said peptide solution and washing each well using PBS (-), 100micro of PBS(-) l which contains bovine serum albumin (it is hereafter called BSA for short) 1% was put into each well, and it was neglected under the room temperature for 1 hour. After washing 3 times by PBS (-), roux rat blood serum 50mul acquired for the preceding clause (1) was put into each well, and it allowed to stand at the room temperature for 1 hour. After washing 3 times by PBS (-), peroxidase-labeling anti-rat IgG50microl was put into each well as a secondary antibody, and it was neglected under the room temperature for 1 hour. After washing twice by PBS (-), The substrate liquid every l [100mu] which dissolved and prepared one o-phenylenediamine (OPD) tablet (a sigma, the catalog number P8287) (10 mg) to 10 ml of 0.1M citrate buffers (pH 4.5) which added hydrogen-peroxide-solution 4mul is dropped at each well, After allowing to stand for 30 minutes under protection from light at a room temperature, the absorbance of 492 nm of each well was measured with the microplate leader (TOSOH, MPR-A4i type). The blood serum with which the rise of antibody titer was checked was used as the anti human HSP47 polyclonal antibody, and was used for the following embodiments.

[0028](3) The buffer system (Laemmli, N. K., "Nature", 283:pp. 249-256, 1970) of the evaluation Laemmli of the anti-HSP47 polyclonal-antibody characteristic by a Western blot technique is used, Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis of the lysate of a HeLa cell was performed in accordance with the following methods. Preparation of concentrating gel was performed as follows. 6.1 ml of distilled water, 0.5M tris (Bio-Rad, catalog number 161-0716) - HCl(pH 6.8) 2.5ml, 10%SDS(Bio-Rad, catalog number 161-0301) 100microl, And 1.3 ml of 30% acrylamide (Bio-Rad, catalog number 161-0101) / N,N'-methylenebis acrylamide (Bio-Rad, catalog number 161-0201) are mixed, Carry out indirect desulfurization mind for 15 minutes, and 10% ammonium persulfate (Bio-Rad, catalog number 161-0700) 50microl and N,N,N',N'-tetramethylethylenediamine. (It is hereafter called TEMED for short) 10microl -- in addition, concentrating gel was prepared (Bio-Rad, catalog number 161-0800). Preparation of resolving gel was performed as follows. 4.045 ml of distilled water, 1.5M tris-HCl(pH 8.8) 2.5ml, 10%SDS100microl, and 3.3 ml of 30% acrylamide / N,N'-methylenebis acrylamide are mixed slowly, It degassed with the aspirator for 15 minutes, and 10% ammonium persulfate 50mul and TEMED5microl were added. As a migration buffer, distilled water was added to 9.0 g of tris, 43.2 g of glycines (Bio-Rad, catalog number 161-0717), and SDS3.0g, it was made 600 ml, and what diluted this with distilled water 5 times was used. A sample buffer 2 ml of distilled water, 2M tris-HCl(pH 6.8) 500microl, What mixed SDS0.32g and beta-mercaptoethanol 800microl and 0.05% (w/v) bromophenol-blue (Bio-Rad, catalog number 161-0404) 400microl was used.

[0029]Under 5% carbon dioxide conditions, at 37 **, the HeLa cell was cultured in the MEM

culture medium which contains inactivation fetal calf serum (it is hereafter called FBS for short) 10%, and the lysate was prepared. After performing SDS-polyacrylamide gel electrophoresis of the obtained HeLa-cell lysate, Gel is stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), The room temperature performed blotting by 100V for 3 hours using protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad). As a blotting buffer. ** 0.025M tris. And the trisglycine buffer which consisted of 0.192M glycine and was adjusted the pH to 8.5 () [Tris Gly Running and Blotting Buffer;Enprotech and] The U.S. Massachusetts state and the buffer which prepared methyl alcohol in addition to catalog number SA100034 so that it might become 20% were used. It blocked by dipping a nitrocellulose membrane in the PBS (-) solution which contains skim milk (Snow Brand Milk Products) 5% for 30 minutes at a room temperature after blotting. Primary antibody reactions were performed after blocking using the screener blotter (sampler tech) by making into a primary antibody the roux rat blood serum acquired for the preceding clause (1). It is said roux rat blood serum 200mul diluted with PBS (-) which contains skim milk (Snow Brand Milk Products) 2% 10 times, and primary antibody reactions were performed for 120 minutes at the room temperature. The shake for 5 minutes by PBS (-) after primary antibody ending reaction using a slow locking shaker 2 times, The nitrocellulose membrane was washed by the PBS (-) solution which contains Tween20 (Bio-Rad, catalog number 170-6531) 0.1% performing shake for 15 minutes, and performing shake for 5 minutes twice by 4 times and also PBS (-). Secondary antibody reactions were performed after the end of washing for 2 hours using 5 ml of solutions which diluted the peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) with the PBS (-) solution which contains skim milk 2% 5000 times. The PBS (-) solution and the PBS (-) solution which contains Tween20 0.1% washed the nitrocellulose membrane under the same conditions as washing after primary antibody reactions after ending reaction.

[0030]After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Western blotting detection reagent; Amersham, catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After settling at a room temperature for 1 minute, an excessive detecting reagent is removed, a nitrocellulose membrane is wrapped in a lap, and the reaction surface was stuck to the X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), and was made to expose. Examination of the reactivity of anti-HSP47 polyclonal antibody was performed after development by measuring the band near [equivalent to HSP47] the molecular weight of 47 kilodalton. The blood serum with which the rise of antibody titer was checked was used for the following embodiments as anti human HSP47 polyclonal antibody.

[0031]Embodiment 2 : culture human breast cancer cell stock MCF7 (ATCC HTB 22) of the measurement (1) Homo sapiens cultured cancer cell of the HSP expression amount of the Homo sapiens cultured cancer cell, In the RPMI1640 culture medium including 10^{-8}Mbeta-

estradiol and the 10% inactivation FBS, it cultivated at 37 ** under 5% carbon dioxide conditions except the time of heat shock processing.

[0032](2) JINSENO side Rg_1 (Matsuura Yakugyo) expressed with said formula (I) that it becomes the last concentration M of 100micro in the culture medium of said human breast cancer cell stock MCF7 two days after JINSENO side Rg_1 processing and heat shock processing seeding was added, and it cultivated for 24 hours. Then, after carrying out heat shock processing for 15 minutes at 45 **, it cultivated at 37 ** all night. The control test was carried out like the above except not adding JINSENO side Rg_1.

[0033](3) Each cell processed for the measurement preceding clause (2) of the HSP expression amount in the Homo sapiens cultured cancer cell was homogenized by the method shown below, and the HSP expression amount was measured in the Western blot technique. Namely, the lysis buffer (lysis buffer) after washing the cell processed for the preceding clause (2) by PBS (-) [1.0%NP-40, 0.15M sodium chloride, the 50mM tris- HCl (pH 8.0), 5 mM-EDTA, 2 mM-N-ethylmaleimide, 2mM phenylmethyl sulfonylfluoride, 2 microg [/ml] leupeptin, and 2 microg [/ml] pepstatin] 1 ml was added and it settled for 20 minutes in Hikami. Then, centrifugality was performed for 20 minutes at 12000 rpm at 4 **. 10micro of supernatant liquid I after centrifugality was added to 790micro of PBS(-) I, and also 200micro of protein assay stain solutions (Dye Reagent Concentrate : Bio-Rad, catalog number 500-0006) I were added. For 5 minutes, after settling at a room temperature, the absorbance was measured at 595 nm and protein quantification was performed. The SDS polyacrylamide gel electrophoresis of the lysate which contains equivalent weight of protein by the buffer system of Laemmli was performed using the sample which performed protein quantification. In accordance with the method described in Embodiment 1, blocking following blotting and it was performed after the electrophoresis. Namely, protein transfer equipment (Trans-Blot Electrophoretic Transfer Cell: Bio-Rad) is used, At the room temperature, gel was stuck to a 0.45-micrometer nitrocellulose membrane (Schleicher & Schuell, catalog number 401196), and blotting was performed 100V for 3 hours. As a blotting buffer, the same thing as the buffer used in said Embodiment 1 (3) was used. After blotting, the nitrocellulose membrane was incubated for 30 minutes at the room temperature in the 10% skim milk (Snow Brand Milk Products)-PBS (-) solution, and nonspecific combination was blocked.

[0034]The anti human HSP47 rat polyclonal antibody manufactured in Embodiment 1 performed primary antibody reactions on the nitrocellulose membrane after blocking. Then, exchange every [a for / 5 minutes], and a solution by PBS (-), and a slow locking shaker performs two washing, Every [a for / 15 minutes] and a solution were exchanged with Tween20 (Bio-Rad, catalog number 170-6531) solution PBS(-)-0.1%, and four washing was performed. Eventually, every [a for / 5 minutes] two washing was performed by PBS (-). A

peroxidase-labeling goat anti-rat IgG antibody (Southern Biotechnology, catalog number 3030-05) after the end of washing, Secondary antibody reactions were performed for 2 hours using 5 ml of antibody solutions diluted and prepared 5000 times with the PBS (-) solution which contains skim milk 2%. After ending reaction, about the nitrocellulose membrane, the solution was changed for 5 minutes at a time with the PBS (-) solution, the solution was changed for 15 minutes at a time with Tween20 solution 2 times and also PBS(-)-0.1%, and the slow locking shaker performed five washing. Finally the PBS (-) solution performed every [a for / 5 minutes] two washing. After removing an excessive PBS (-) solution, a Western-blotting detecting reagent (ECL Westernblotting detection reagent;Amersham and catalog number RPN2106) is sprinkled on a nitrocellulose membrane, After incubating for 1 minute, the excessive detecting reagent was removed, the nitrocellulose membrane was wrapped in the lap, the reaction surface was stuck to the X-ray film (Kodak X-OMAT, AR, catalog number 165 1454), was exposed, was developed, and the existence of HSP47 was examined.

[0035]In breast cancer cell line MCF7 which did not add a control test, i.e., JINSENO side Rg_1, the band of molecular weight abbreviation 47kD was detected one. In breast cancer cell line MCF7 which added JINSENO side Rg_1, the corresponding band was a very thin band. That is, JINSENO side Rg_1 can be concluded to have the activity of the synthetic inhibitor which controls the manifestation of HSP47, and this fact shows that JINSENO side Rg_1 works restrainedly to sthenia of extracellular matrix production.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing JINSENO sides as an active principle.
[Claim 2]Synthetic inhibitor of a heat shock protein with an according to claim 1 molecular weight of 47 kilodalton whose JINSENO sides are JINSENO side Rg_1.
[Claim 3]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing an extract of vegetation containing JINSENO sides as an active principle.
[Claim 4]Synthetic inhibitor of a heat shock protein with a molecular weight of 47 kilodalton containing an extract of a ginseng or Ginseng Radix Rubra as an active principle.

[Translation done.]